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# RECENT ADVANCES IN PLANT PHYSIOLOGY

BY

E. C. BARTON-WRIGHT

M.Sc.(Lond.), F.R.S.E.

*Chief Assistant at the Scottish Society for Research in Plant Breeding,  
Corstorphine, Edinburgh*

*Formerly Lecturer in Botany in the University of London,  
King's College*

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TO  
MY FRIEND  
JAMES MONTAGU DRUMMOND, M.A., F.L.S.  
PROFESSOR OF BOTANY IN THE  
VICTORIA UNIVERSITY OF MANCHESTER

*Printed in Great Britain*

## PREFACE TO THE SECOND EDITION

IN spite of its manifest defects, the early call for a second edition of this book in these times of acute trade depression is very gratifying. The text has been revised and brought up to date, and I have to thank numerous correspondents in this country and in America for pointing out mistakes, ambiguities and typographical errors. I am especially indebted to Dr. Eric Ashby of the Imperial College of Science, South Kensington, for his help in many directions, and also to my colleague, Dr. V. E. McM. Davey, for his invaluable assistance. To Miss M. Jockel of the Royal Botanic Garden, Edinburgh, I owe a world of gratitude for carrying out the wearisome task of reading the whole of the proofs and checking the references. I should also like to take this opportunity of thanking Messrs. Churchill for the ample facilities they have given me for revision of the text.

E. B-W.

CORSTORPHINE.

## PREFACE TO THE FIRST EDITION

THE present text covers a strictly limited period ; approximately the years 1918 to 1928. An attempt has been made, however, to bring the subject-matter up to date, and some of the more relevant papers published during the present year (1929) have also been included.

Two choices were open to the author when he commenced this book : either to throw down before the reader a mass of names and references to the literature, or to discuss critically and in detail a few important and fundamental papers published in each year of the last decade. After a good deal of hesitation, the latter alternative was chosen. It will be seen that the bibliography is not extensive and makes no pretence to being exhaustive. The precise meaning that should be attached to the term " important " paper is largely one of personal taste, and the old adage " One man's meat is another man's poison " applies as well here as in other matters. The author has based his choice of papers entirely on his own personal predilections, and it is for others to say whether his choice be poor and indifferent, or if he has succeeded in steering a medium and reasonable course.

This book has been especially prepared for students, and not for specialists, and the needs of the former have been principally considered. There is at the present time no adequate text-book which covers the ground necessary for students reading for their final honours degree, and it is hoped that the present book fills the gap.

Stress has been laid on the economic aspects of the subject. Unless botanical laboratories are prepared to recognise the enormous economic possibilities of their subject, progress will be quickly stayed. The great advances plant physiology has made in America, with its separate societies and journals, is undoubtedly largely due to the fact that the economic, and not the purely

academic, side has been principally exploited. Agriculturists in this country have been slow to recognise the great importance to them of plant physiology. This state of affairs is not to be wondered at, and the blame must be laid at the door of plant physiologists. The plant physiologist, until comparatively recently, has been content to work on purely academic problems and has left the wider economic issues alone. Research for research's sake is an admirable slogan, but it has its limitations, and in the economic conditions at present prevailing in this country it is but natural that pure research with no economic outlook or possibilities is not held in such awe as in pre-war days. So little is the practical importance of plant physiology recognised here and in the Colonies that the author well remembers being told by a high Colonial agricultural officer: "Plant physiology; why, that is an amusing hobby!" Yet how many would care to stigmatise the work of Balls on the cotton plant in Egypt, or Denham and the work of the Shirley Institute in this country, as an "amusing hobby"? After all, the final explanation of the workings of the living organism must be given in terms of physics and chemistry, and that is the ultimate goal of physiology.

In the past plant physiology has occupied the "Cinderella" position in the botanical world. But the very active investigations that have taken place since the war have shown that many problems which appeared to be simple on the surface are in reality highly complex, and that plant physiology is not merely the contemplation of the obvious, as many have supposed. The whole situation, however, is now altered, and the very rapid strides that have been made in this branch of plant study have frequently brought forward the suggestion that it should be severed as a separate subject from botany. Such a separation would have many advantages, but it is probably outweighed by the disadvantages. It was an evil day for zoology when animal physiology was removed from it as a separate subject; and certainly botanical science would suffer from such a divorce.

There is a point which requires special emphasis here. Our present knowledge of plant metabolism is very seriously deficient in several directions. This unfortunate state of affairs is due to

several causes, among the principal of which is the fact that in plants several complex chemical reactions take place within the compass of a single cell, which makes the matter difficult of investigation. Nevertheless, the difficulty is intensified by the sporadic invasions of organic chemists into a domain of which they have little or no knowledge, with ready-made explanations based on *in vitro* experiments which are probably remote from the chemical reactions of the living plant. It is difficult to know why botanical physiology should be made the general playground for the imaginative theorising of persons who have no very reliable knowledge of the living plant.

The schools of plant physiology, founded by Professor V. H. Blackman at South Kensington and Dr. F. F. Blackman at Cambridge, have done an immense service in bringing the subject into prominence in this country. The work produced from their laboratories has always been of the highest type, and they and their co-workers, Dr. Knight and Dr. Gregory at South Kensington, and Dr. Briggs, Dr. Kidd and Dr. West at Cambridge, to mention but a few, have laid plant physiology under an immense burden of debt for their pioneer labours.

It is a pleasure to acknowledge the help I have received from numerous friends in the preparation of the text. To my wife and Mrs. V. E. Kastner I am indebted for reading the manuscript and for the preparation of some of the figures. I am very grateful to Professor V. H. Blackman, Professor Tansley, the Council of the Royal Society, Messrs. Longmans, Green & Co., and a number of authors and publishers for allowing me to use illustrations from their published works. Mr. C. S. Semmens is responsible for the preparation of the majority of the figures, and I have to thank him for his untiring help. Lastly, to Messrs. J. & A. Churchill I owe a debt of gratitude for the very generous way in which they have always been ready to place their expert advice at my disposal.

BOTANY DEPARTMENT,  
KING'S COLLEGE, STRAND.

E. BARTON-WRIGHT

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# RECENT ADVANCES IN PLANT PHYSIOLOGY

## CHAPTER I

### ABSORPTION OF WATER AND TRANSPIRATION

*The Root System of Plants—Absorption of Water by the Root—Suction Pressure—Measurement of Suction Pressure—Suction Pressure and its Magnitude—Transpiration—Cuticular and Stomatal Transpiration—Factors Affecting Transpiration Rate—Relative Transpiration—Stomatal Regulation of Transpiration—Mechanism of Stomatal Movement—Effect of Light on Transpiration from the Mesophyll—Transpiration of Xerophytes—Importance of Transpiration to the Plant.*

#### The Root System of Plants

It is unfortunate that the root system of plants has not received the attention of the aerial portions at the hands of the physiologist. This is no doubt due to the fact that it lies underground, and is therefore of difficult accessibility and at the same time does not lend itself very readily to experimental treatment. Certainly this highly important region is of great physiological importance, for it is the moisture- and salt-absorbing region of the normal green plant, and at the same time acts as an anchorage for the plant to its substratum.

An important ecological and physiological investigation of different root systems has been conducted by Weaver (1919, 1920, 1926), and the following is mainly a summary of this work: The method employed was to dig trenches 2 to 3 feet wide and 6 to 10 feet long to a depth of about 6 feet by the side of the plant to be examined. This offered an open face on which to work, and by careful digging with a hand-pick the root system could with

## 2 ABSORPTION OF WATER AND TRANSPIRATION

practice be removed in its entirety. As the work proceeded the trench was deepened, often to a depth of 20 feet or more. The root systems were removed and photographed or sketched on an exact scale.

In the prairies of Nebraska some thirty-one perennial plants were investigated. *Panicum virgatum*, although it showed a preference for loose sandy soils, grew abundantly in many positions throughout the prairies. The roots of this plant are very coarse and the longest of any grass that was investigated. Several reached a vertical depth of 8.5 feet. In the first 6 or 7 feet there was little branching and laterals only occurred sparingly. In *Andropogon furcatus* there is an abundant root system which grows vertically and obliquely downward and forms a dense sod. The greatest depth reached by the roots is 6 feet 10 inches, and all the roots show profuse branching. *A. scoparius* extends to a depth of about 5 feet in sandy soil. In gravel soil mixed with sand and a rocky subsoil of decayed sandstone the roots only reached to a depth of 3 feet. With clay loam soil and clay subsoil the length of the roots only extended to 28 inches, whereas in clay loam alone the roots extended to 65 inches. *A. nutans* is a deep-rooted form. The maximum depth of root is found to vary between 51 and 59 inches. There is very little lateral expansion, and the roots completely occupy the soil by profuse branching to the second and third order. Other grasses investigated were *Stipa spartea* (21 to 26 inches), *Koeleria cristata* (15 inches), *Elymus canadensis* (16 to 22 inches), *Agropyrum repens* (2 to 3 feet), *Distichlis spicata* (18 inches to 2 feet), an important inhabitant of alkaline soils which is of considerable forage value, *Sporobolus longifolius* (17 to 40 inches), which occupies a wide area and the roots of which are very dense.

Of the other perennials investigated, *Solidago rigida* possesses a root system which spreads immediately below the soil surface to a radius of 12 to 18 inches on either side of the plant. The roots are very abundant for the first 2 feet of the soil, and the maximum depth that was ever found was 5 feet. *Solidago canadensis* behaves in the same way as *S. rigida* and branches freely below the surface, but often reaches as great a depth as 9 to 10 feet.

The most obvious conclusion to be drawn from a consideration of these data is the fact that prairie plants are provided with a well-developed, deep-seated and extensive root system. Weaver divided the thirty-three species that were examined upon a basis of root depth into three classes :—

(I.) Shallow-rooted plants not extending below the first 2 feet of soil. This class consists wholly of grasses, such as *Koeleria cristata*, *Stipa spartea*, *Elymus canadensis*, *Distichlis spicata*, *Sporobolus longifolius*, and *Aristida oligantha*.

(II.) Plants with roots extending well below the second foot of soil, but seldom deeper than 5 feet. Here belong : *Andropogon scoparius*, *A. nutans*, *Bouteloua gracilis*, *Bulbilis dactyloides*, *Verbena stricta*, *Helianthus rigidus*, *Solidago rigida*, *Petalostemon candidus*.

(III.) Of the plants placed in this section, 55 per cent. have roots which extend beyond a depth of 5 feet ; indeed, most of them to depths of 7 to 9 feet and a maximum depth of from 13 to 20 feet or more. They may therefore all be classed together as deep-rooted species. The following are placed in this class : *Panicum virgatum*, *Andropogon furcatus*, *Agropyrum repens*, *Solidago canadensis*, *Astragalus crassicaarpus*, *Psoralea tenuiflora*, *Lygodesmia juncea*, *Ceanothus ovatus*, *Baptisia bracteata*, *Lespedeza capitata*, *Glycyrrhiza lepidota*, *Brauneria pallida*, *Vernonia Baldwini*, and *Kuhnia glutinosa* (Fig. 1).

The cause of the remarkable root development of prairie plants can be obtained from a study of prairie environment. The plants inhabiting such regions live under semi-arid climatic conditions in which water supply is

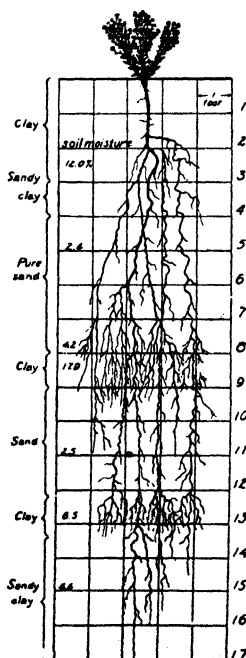


FIG. 1.—The root system of *Kuhnia glutinosa*, showing the deep penetration in prairie soil. (After Weaver, *Ecological Relations of Roots*.)

#### 4 ABSORPTION OF WATER AND TRANSPIRATION

the chief limiting factor for growth. During certain portions of the growing season extreme xerophytic conditions are brought to bear on the vegetation. The water content of the soil is brought to the non-available point as far as a depth of 4 to 5 feet in certain years, and especially when the evaporating power of the air is high. It is in response to these environmental conditions that many species have developed extensive root systems. For just as the evaporating power of the air and the nature of the transpiring organs determine the water loss, so the soil moisture and the nature of the root systems determine the supply. Thus great root depth is to be correlated with deep soil moisture. The nature of the soil also has a marked effect upon root development. Several species, including grasses, penetrate to a depth of 2 to 3 feet in loamy soil, but to a lesser depth in the same type of soil when it is overlaid with a hard subsoil of clay.

The chaparral community lying between the Ohio-Missouri deciduous forest complex and the prairies to the westward was also investigated. Here *Symphoricarpos*, *Rhus*, *Corylus* and *Rosa* play the part of dominants. *Symphoricarpos vulgaris* forms dense shady clumps which exclude even the tolerant *Poa pratensis*. The maximum root depth reached by this plant was 65 inches, but the lack of linear extension is made up by a remarkably well-developed absorbing surface. *Rhus glabra* is never found to extend to a depth of more than 80 inches. In *Corylus americana* the roots extend to great depths, 10 to 11 feet, and in *Rosa arkansana* the tap root reaches the greatest depth of all these species, and grows vertically downward for 21 feet 2 inches.

It will be seen that all the members of the chaparral community possess well-developed absorbing systems, which, though showing variability in their length of vertical extension, are always deep-seated. They are all well adapted either by means of above-ground or under-ground rhizomes or root offshoots to invade effectively, if slowly, prairie sod. *Symphoricarpos* extends its area by above-ground stems as well as by those below the surface. The above-ground stems furnish the more rapid method of

migration, but they frequently fail utterly to become rooted in prairie sod. The under-ground rhizomes, however, supply a more certain method of establishment. Once this shrub has established itself, it quickly reacts on the habitat by modifying the composition of the soil and increasing the water content of the air. The presence of shrubs decreases wind movement, and their shade reduces light and temperature. These factors help to decrease the evaporation of water from the soil surface and conserve the moisture of the soil. The run-off is greatly reduced as a result of the rich mulch of fallen leaves and large quantities of wind-transported *débris* of plants which become lodged among the stems. Drifts of snow also find lodgement between these shrubs, and when they melt, add considerably to the soil moisture. In a few years, from the addition of vegetable matter to the soil, considerable quantities of humus fill the former prairie soil. *Rhus* has very similar effects upon the habitat to *Symphoricarpos*, but since it is a taller shrub its action on the bordering grassland is more pronounced.

The prairies of south-eastern Washington and adjacent Idaho represent an extreme western extension of the great grassland formation lying east of the Rocky Mountains. Here *Agropyrum spicatum*, *Festuca ovina ingrata*, *Koeleria cristata* and *Poa Sandbergii* are dominants. *Stipa* and late blooming grasses are absent.

The distribution of rainfall probably accounts for the latter fact.

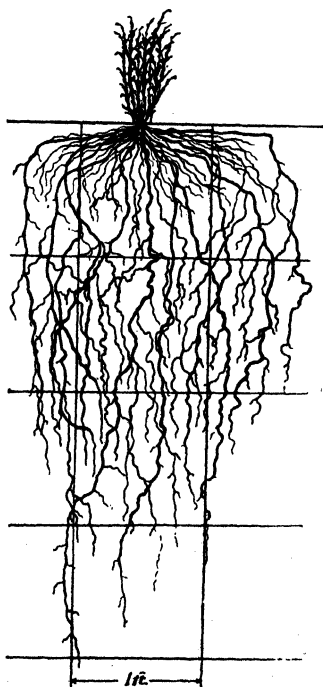


FIG. 2.—*Artemisia frigida*; an example of a root system of the plains association. (After Weaver, *Ecological Relations of Roots*.)

## 6 ABSORPTION OF WATER AND TRANSPIRATION

During the whole growing season the rainfall is 5 inches, and except for the highly retentive loam-silt soil, the region is practically desert. The maximum depth of penetration of roots is shown by *Agropyrum spicatum* (4 feet 10 inches), while the other dominants, *K. cristata*, *P. Sandbergii* and *F. ovina ingrata*, are all shallow-rooted plants, and the bulk of their absorbing system lies above the 18-inch level.

The root system of plants living on the plains also shows a deep penetration. In only a few cases do they have an extension of less than 2 feet into the soil. Nevertheless, they are not found to reach the depths attained by the prairie flora. Most of the species dwelling on plains show, in addition to their deep-rooting system, a fine system of roots near the surface with wide-spreading laterals (Fig. 2).

The annual rainfall in such regions is generally small (15 inches), and the major portion of this precipitation falls during the growing season. Such a seasonal distribution of rainfall is very favourable for the growth of grasses. The soil moisture of the plains is not high, and in some cases it was found that the soil may be uniformly dry for as great a depth as 7 feet. It is possible that it is to these factors that the deep root penetration of plants inhabiting plains is due. ✓

The climatic factors of the sandhills area near Colorado are very similar to those of the plains. The root system is not deeply developed. Eight of the nineteen species investigated had roots which were almost entirely confined to the first 2 feet of soil. Even the deep-rooted forms, such as *Eriogonum microthecum* and *Artemisia filifolia*, show an excellent development of laterals in the surface layer. In the soft substratum of the sandhills such a marked development of lateral root growth cannot be attributed to mechanical hindrance to penetration, but is probably connected with the water supply. Any rain is immediately absorbed and there is no run-off. As soon as the rain has ceased, evaporation dries the surface soil with great rapidity, but evaporation does not proceed to any great depth. The surface layer of dry soil thus formed acts as a mulch and retards any further evaporation. At a depth of a few inches below the surface the sand is always found

to be moist, and can even be moulded into lumps with the hand. Covering vegetation is also scarce and loss of water from transpiration correspondingly small (Fig. 3).

The root system of plants growing at an altitude of 8,000 feet in the Pike's Peak region of the Rocky Mountains was also investigated. Here the soil is composed of disintegrated granite, and the degree of disintegration and decomposition largely determines the type of plant community occupying any particular area.

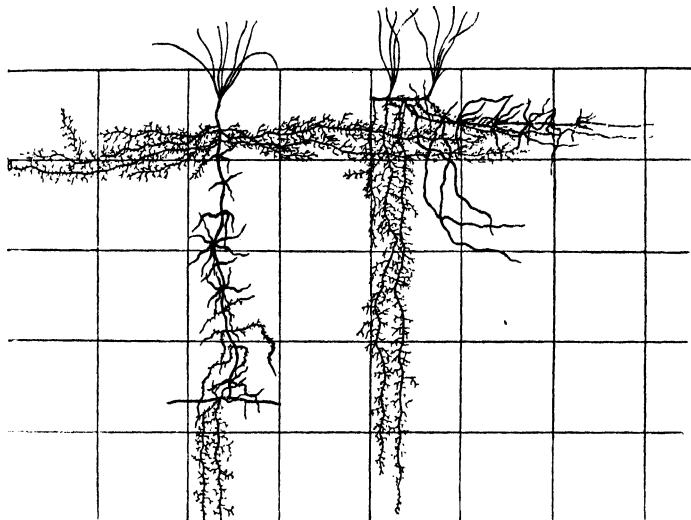


FIG. 3.—*Redfieldia flexuosa*, showing the distribution of rhizomes and roots in the sandhills subclimax association. (After Weaver, *Ecological Relations of Roots*.)

All the plants inhabiting this area possess roots well adapted to secure moisture and nutrients from the surface area of the soil. The roots are characterised by a shallow widely spreading system practically confined to the first 18 inches of the soil and showing the best development in the first 12 inches. The lack of depth extension is compensated by lateral extension and profuse branching. These adaptations find a ready explanation from the nature of the soil and the distribution of rainfall.

The soils of the gravel-slide consist of a superficial layer com-

## 8 ABSORPTION OF WATER AND TRANSPIRATION

posed of coarse angular rock particles which vary in size from fragments of over an inch to a few millimetres in diameter. Except during periods of rain, the surface is very dry, and as the slope is steep, there is often a constant movement of rock particles down the mountain side. Most of the plant tops were found to have slipped down the slope from 2 to 8 inches or more. This surface layer is very efficient in preventing any run-off, and at the same time forms a dry mulch which protects the soil from excessive

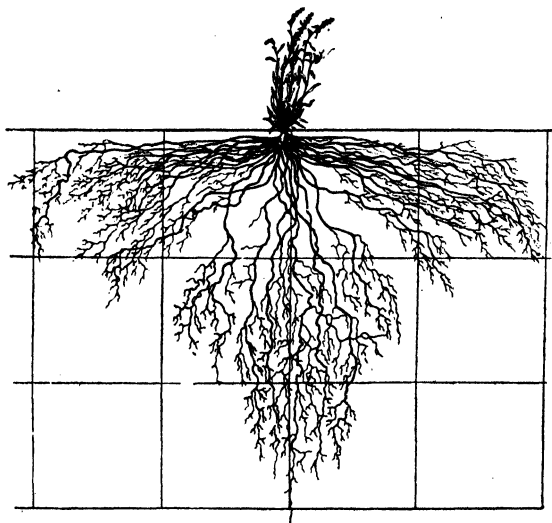


FIG. 4.—*Solidago oreophila*, a plant of the half-gravel-slide community, showing wide-spreading lateral and deep central roots. (After Weaver, *Ecological Relations of Roots*.)

evaporation. It was found that the soil moisture was at no time very great, but was evenly distributed throughout the first 18 inches of soil. The evaporating power of the air in this region is high, no doubt partly due to the considerable air movements of such a high altitude. The root system is clearly a response to environment, and the large number of roots which run up the slope serve in part as an effective anchorage (Fig. 4).

The half-gravel-slide community represents a distinct successional advance over that of the gravel-slide and forms an inter-

mediate stage between the gravel-slide and the forest. Wide lateral expansion of the root system is prominent in this situation, and at the same time is supplemented by a deep-seated portion which extracts moisture and nutrients from below the 18-inch level, as well as from the second and third foot of soil. The spreading surface roots may be explained by the frequent occurrence of mountain showers, which give a constant supply of water to the shallow soils. The thick surface layer of loose rock frag-

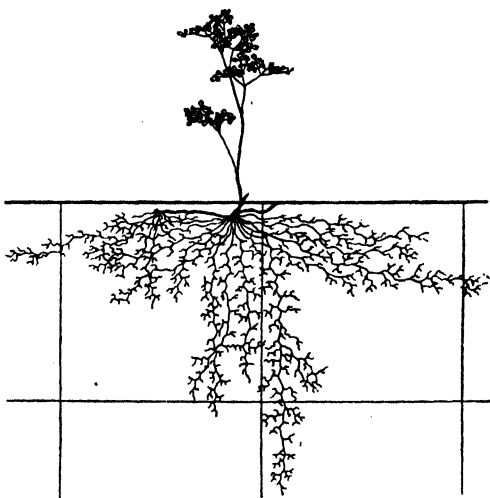


FIG. 5.—*Thalictrum Fendleri*, showing the roots extending practically parallel with the surface of the soil with small penetration in depth. (After Weaver, *Ecological Relations of Roots*.)

ments of the gravel-slide are here more disintegrated and closely packed, and this soon gives way to the true soil. Hence evaporation of water is free to take place, and this factor combined with the competition of the taller half-gravel-slide plants accounts for the disappearance of the gravel-slide species.

The final occupants of a half-gravel-slide are a forest community. A number of herbs and undershrubs characteristic of the more mesophytic type of forest, such as *Pirola chlorantha*, *Thalictrum Fendleri*, *Erigeron macranthus*, *E. asper*, *Ribes lacustre*,

## 10 ABSORPTION OF WATER AND TRANSPIRATION

*Rosa acicularis*, *Senecio cernuus* and *Heuchera parvifolia*, were examined. The bulk of the herbs and shrubs of this forest floor are relatively shallow-rooted. Almost without exception the mass of the absorbing system lies within the first 18 inches of the soil surface. Even the roots of the Douglas fir and spruce trees were found to possess many shallow roots. The forest soil is usually deeper and contains more organic matter than the half-gravel-slide. One or two inches of duff is generally found, and beneath this layer the soil is particularly rich in humus to a variable depth of 8 to 18 inches. Such a substratum furnishes an excellent medium for holding winter rain as well as the frequent summer showers. The shade of the trees reduces the evaporating power of the air, and the loss of water from the soil is further lessened by the layer of duff. The entering water does not penetrate the soil deeply, and the greatest amount of available water is to be found in the first 18 inches of soil. This doubtless accounts for the shallow root habit exhibited by forest shrubs and herbs (Fig. 5).

A number of polydemic species were also investigated, each growing on at least two different habitats. The theory put forward by Weaver, that water content of the soil determines the nature of the root system, gained considerable support from these cases. Thus *Bouteloua*, *Stipa* and *Chrysopsis*, when growing on the plains, were all found to be deep-rooted, and sometimes extended to a depth of 13 feet. When growing on the sandhills they conformed to the root-habit of most of the plants growing in this situation. None of them reached to more than half their former depth, and all showed a marked development of shallow lateral roots. Others, however, such as *Allionia linearis* and *Abronia fragrans*, growing in these same habitats were only very slightly modified. *Euphorbia* and *Yucca* were very frequently modified when grown in half-gravel, although at the same time conforming to the root system; they exhibit when grown on the plains a rather deep and wide-spreading absorbing system. In the former, the extension in depth was always much less, while the branching, like *Yucca*, was much more pronounced (Figs. 6 and 7).

Weaver considered that the water-content of the soil offers a logical explanation for this community root habit. In general, root position conforms in a striking manner to the distribution of moisture in the soil, and throughout the whole of this very elaborate investigation only a very few species were discovered,

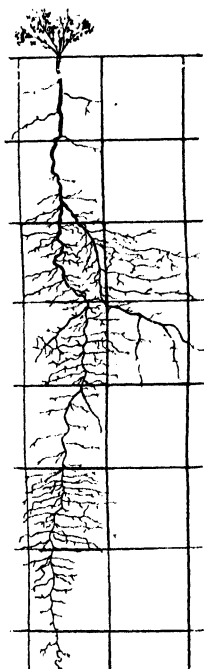


FIG. 6.—The root system of *Euphorbia montana* from the plains. (After Weaver, *Ecological Relations of Roots*.)

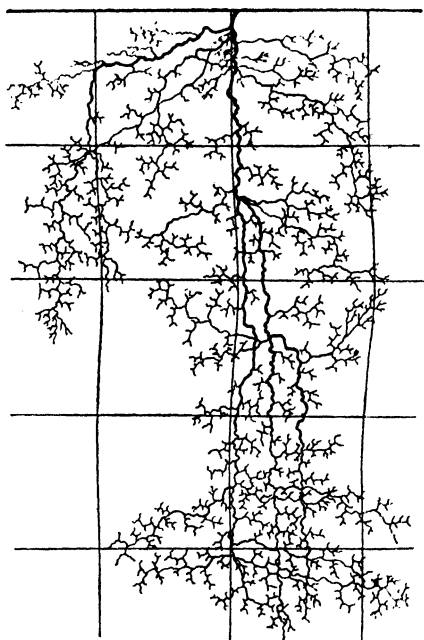


FIG. 7.—The root system of *Euphorbia montana* from the half-gravel-slide. (After Weaver, *Ecological Relations of Roots*.)

which showed little or no variation of root system when growing under different external conditions. Since root position so clearly reflects the moisture condition of the soil, especially when interpreted in its community relations, the study of the root systems of plants will greatly increase our knowledge of the value of different plants in indicating lands of agricultural or non-agricultural value.

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Weaver, Jean and Crist (1922) have further extended this work to the root system of crop plants in different types of soil moisture, aeration, etc. The stations chosen were Peru in Eastern Nebraska (rainfall 33 inches) ; Lincoln, Nebraska (28 inches) ; Phillipsburg, Kansas (23 inches) ; and Burlington, Colorado (17 inches). These stations form a decreasing series in rainfall and relative humidity of the air, and so correspond respectively with subclimax prairie, true prairie, mixed prairie and shortgrass, and pure shortgrass vegetation. A remarkable correlation was found between the root systems of the crop plants and the natural vegetation. The penetration of the crop plants was extensive. In one case recorded by these observers of a strain of heavy-cropping Kherson oats, the roots penetrated to a depth of over 6 feet in Peru, but the maximum development of rootlets occupied the top foot of soil. In Phillipsburg, barley reached a depth of over 6 feet, while *Medicago sativa* (alfalfa) and *Melilotus alba* (sweet clover) extended to approximately 5 feet. At Burlington, owing to a hard pan formed by colloids and carbonates in the sub-soil, root extension was prevented and consequently less development was found. The usual depths for the roots to descend were ascertained to be about 2 or 3 feet, and at the same time there was considerable lateral development.

In the light of their investigations these authors considered that the statements made in agricultural literature to the effect that only the surface soil (about 8 inches) layer is used by roots for absorption, and that the lower subsoil only plays an indirect part, need to be very considerably revised. In fact, in nearly every case, where the roots of crop plants were excavated, the sum total of the development below the cultivated soil surface layer was as big, and in some cases even bigger, than in the surface soil. The dependence of the plant on these more deeply seated layers becomes prominent in times of drought. At such times the vegetation remains unwithered, and fair yields are still obtained from the crops when the surface layer is practically exhausted of moisture.

To determine the depths at which nitrates and water were still absorbed by roots, experiments were carried out by growing crop

plants in special containers with wax seals at different levels, and the well-compacted soil at each particular level occupied the same relative level as in the field. Oats were found to absorb water at depths of 2·5 feet, and the amount of water absorbed by barley was in direct proportion to the downward extension of the root system. *Zea Mays* was discovered to absorb water to a depth of 3 or 4 feet of soil, and to absorb smaller amounts after a depth of 5 feet. Considerable quantities of nitrates were removed by roots from the same levels. In every case in which the roots came in contact with a fertilised layer they showed extensive development and branched vigorously, but normal extension into the deeper layers was checked. An important general conclusion arrived at by the authors is that manuring the surface layers of soil where precipitation is small and conditions of drought are ever present is detrimental to crop growth.

### Absorption of Water by the Root

In the case of land plants it is only a special portion of the root that can absorb water and dissolved salts, namely, the root hairs. It is often stated that the function of the root hairs is to absorb water. This is certainly true, but they also possess another important function, inasmuch as they increase the area of absorption of the root.

The root hairs are delicate structures which grow out from the piliferous layer of the root. They have a more or less gelatinous coating on their walls by means of which they are able to adhere to the particles of the soil and make intimate contact with them. They do not ordinarily occur over the entire surface of the root. The terminal portion of the root, consisting of the root cap and the region of cell division and cell enlargement, does not bear root hairs. The older portions of the root are also devoid of hairs. Generally speaking, the root hairs are for the most part confined to a limited zone of about 1 to 4 cm. in extent near the tip of the root. (For a full review of the literature dealing with the structure and physiology of root hairs, see Farr, 1928.)

Till the important publication in 1918 by Thoday, it was

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considered that the root hairs withdrew water from the soil by virtue of their osmotic pressure. This, however, is not the case. The entrance of water into the root hairs depends upon what has been called by Ursprung and Blum (1916) *suction force*, while Stiles (1922) has termed it the *suction pressure* of these cells.

If a cell isolated from its surroundings be placed in water, water will enter and distend the cell wall. The wall is elastic, in that it is able to resist extension. The distended wall compresses the protoplasm and cell sap, with the result that the consequent hydrostatic pressure, usually termed the *turgor pressure*, is equal and opposite at any moment to the inward components of the

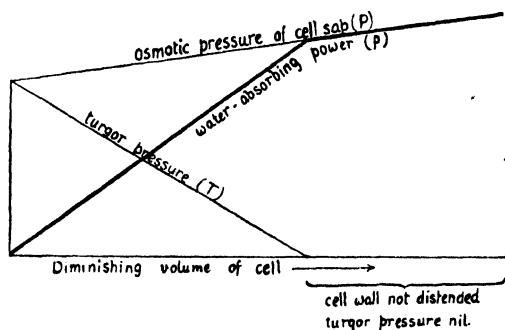


FIG. 8.—Curves illustrating the relationship between the osmotic pressure of the cell sap ( $P$ ), turgor pressure of the cell wall ( $T$ ) and suction pressure of the cell ( $p$ ). (After Thoday, *New. Phyt.*)

tensions of the cell wall. This turgor pressure tends to force water out of the cell. When the swelling is complete and equilibrium reached, the turgor pressure completely balances the osmotic pressure of the cell sap. In this condition the cell is fully turgid and it is in equilibrium with water, and for this reason is incapable of absorbing more water. This condition will last as long as either the osmotic pressure or the tension of the cell wall suffer no change. If this turgor pressure be represented by  $T$ , and the osmotic pressure by  $P$ , then under the conditions of equilibrium discussed above :—

$$P = T$$

or

$$P - T = 0$$

The value  $(P - T)$  is known as the suction pressure of the cell. It is the part of the osmotic pressure left over to suck water into the cell and gives a measure of the water-absorbing capacity of the cell. This suction pressure is fundamental in all processes connected with the water-absorbing power of root hairs.

The connection between the osmotic pressure, turgor pressure and suction pressure of a cell is shown in Fig. 8. It will be seen from the nature of the curve that with diminishing turgor pressure there is a corresponding rise in the suction pressure.

In the case of a fully flaccid cell, the turgor pressure is zero. The osmotic pressure is equal to the suction pressure, and the water-absorbing power of a fully flaccid cell depends on its osmotic pressure. From this discussion it will be understood that the

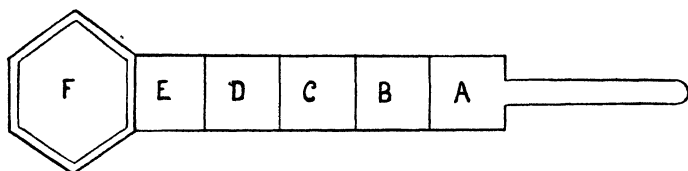


FIG. 9.—Diagrammatic representation of root hair, A, in connection with cortical cells B to E, and trachea F. (After V. H. Blackman, *New. Phyt.*)

older view that it was the osmotic pressure of the cell which played an all-important part in the absorption of water is erroneous.

**Condition of Equilibrium between Adjacent Cells.** Since the water-absorbing power of a cell is dependent on its suction pressure, the question arises as to how far this will affect the situation between two cells in contact with one another. If these cells are represented by A and B, we have as the condition of equilibrium :—

$$(P_A - T_A) = (P_B - T_B)$$

If the value  $(P_A - T_A)$  be greater than  $(P_B - T_B)$ , then A will absorb water from B, irrespective of the absolute values of  $P_A$  and  $P_B$ . It is quite possible, for example, to have B with a greater osmotic pressure than A, yet, because the turgor pressure is also greater, it may be quite unable to absorb water from A, and, in fact, may yield water to it. Similarly, as V. H. Blackman (1921)

has pointed out, if a root hair be in contact with a number of cortical cells and finally a xylem vessel (Fig. 9), A, B, C, F and a gradient of suction pressure be established, water will enter the root hair A, pass into the cortical cells, and finally enter the trachea. A gradient of suction pressure all the way is required. On the assumption that the root hair A is exposed to water and has the highest osmotic pressure, the cells B to F having progressively lower pressures, water will still pass from A to F. As A takes up water, its absorbing power will fall below B, and B will begin to take up water from A. In the same way as the absorbing power of B falls below the osmotic pressure of C, C will take up water from B. As the cells of the root hair and cortex become more and more turgid and cease to have any absorbing power, F will be able to draw water from outside A, however low be its osmotic pressure, and the force with which this water will be drawn in will depend absolutely on the difference between the osmotic pressure of the solution external to A and the osmotic pressure of F. The presence of the intermediary cells can be neglected, though their interposition will naturally cause a reduction in the rate of flow. ✓

**Measurement of Suction Pressure.** Two distinct methods have been described by Ursprung and Blum (1916, 1918, 1919, 1920) for the determination of suction pressure. Since the suction pressure represents the osmotic pressure of the cell minus the turgor pressure of the cell wall, Ursprung and Blum hit on the idea of directly employing plasmolysis for this purpose. The actual estimation was carried out as follows: In the first stage it was necessary to measure the volume of the cell, and since this could not be effected in water or salt solutions, it was carried out in liquid paraffin. The second stage consisted in finding a cane sugar solution that just did not change the volume of the cell, and lastly, one that just did. The mean of the two values gave the suction pressure of the cell.

✓Ernest (1931) has pointed out a serious error in this method. The removal of a portion of a plant organ and its immersion in paraffin oil will at once reduce the supply of water to and the loss from the tissues to zero. The gradients of suction pressure

which cause water movement from cell to cell are dynamic and not static gradients, and if the supply of water through the cells be stopped, a dynamic equilibrium will no longer be present and the cells will begin to lose water to one another until the same suction pressure is present in all. Since the dynamic relationships of the cells, on which their suction pressure gradient depends, will be completely deranged the moment they are removed from the plant, and, as in paraffin, there will be neither gain nor loss of water from the cells, the cells will naturally pass into a state of static equilibrium and in a short time will all attain the same suction pressure. A further difficulty that arises in this connection is that Ursprung and Blum state that they only worked with intact cells, as it would be impossible to measure the suction pressure of a ruptured cell. In the case of two adjacent cells with the same suction pressure, if one of the cells suffers disruption its contents will be able to exert their full osmotic pressure. This osmotic pressure will be greater than the suction pressure of the intact cell, and its contents coming into contact with the latter will draw water from the uninjured cell, and as a consequence its suction pressure will be increased. By the use of stripped portions of tissue instead of sections, this difficulty may be overcome.

The second method of Ursprung and Blum consisted in determining the concentration of a solution which contained some substance to which the cell membrane was impermeable and caused no change in the volume of the cell. Here the value of the suction pressure would be zero, and if  $P'$  represents the osmotic pressure of the external solution,  $P$  the osmotic pressure of the cell sap, and  $T$  the turgor pressure of the cell wall, we have :—

$$\begin{array}{l} P - P' - T = 0 \\ \text{or} \qquad \qquad \qquad P' = P - T \dots\dots\dots (a) \end{array}$$

If  $S$  represents the suction pressure, when the cell is placed in distilled water :—

$$S = P - T \dots\dots\dots (b)$$

From equations (a) and (b) it follows that  $P'$  and  $S$  are both equal

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to  $(P - T)$  and the value of  $T$  is the same in both equations, thus :—

$$S = P'$$

Hence the suction pressure of a cell is equal to the osmotic pressure of some non-penetrating solution in which the cell remains unchanged in volume. As a matter of fact, in actual practice, it is better to use weight rather than volume for the estimation. To find the suction pressure of the cells of some given tissue by this method, all that is necessary is to take varying concentrations of cane sugar solution and immerse equal weights of the tissue in them for a given time (usually two hours). The tissue is then removed and weighed. The osmotic pressure of the concentration which brings about no change in weight of the tissue gives the value of the suction pressure of the cells.

Molz (1926) has devised a very simple method of estimating suction pressure. Cane sugar solutions of varying concentrations were placed in small bottles of 20 c.c. capacity. The plant organ, or part of the organ, after severance from the plant, was placed in paraffin oil, in which it could remain for several hours without injury. Appropriate strips were then cut from the material under paraffin. The strips were so cut that their length could be determined exactly by means of a scale on a microscope slide and a micrometer eye-piece. The length of the strips was determined and the paraffin was quickly removed with a piece of filter paper, and they were placed in the bottles with cane sugar solution for one to one and a half hours. The lengths were measured again in the strength of cane sugar solution in which the strip had been immersed. The osmotic pressure of the cane sugar solution which caused neither increase nor decrease in length of a strip gave the suction pressure of the tissue.

**Suction Pressure and its Magnitude.** The first really adequate measurements of suction pressure were made by Ursprung and Blum (1916, 1921). Working with the roots and leaves of *Fagus sylvatica* they discovered considerable differences in the value of the suction pressure in different parts of the same organ. In general terms the suction pressure rose from the guard-cells,

through the spongy tissue to the palisade parenchyma in the leaf. In the stem, the gradient of pressure was from within outwards, and in the root from the piliferous layer inwards; in fact, the reverse of the stem. They found in *Vicia Faba* and *Phaseolus vulgaris* that the suction pressure of the cells of the roots in both species showed a steady increase from the piliferous layer as far as the innermost layer of the cortex which abutted on the endodermis, and the suction pressure of the latter was very much less than that of the cortical tissue, but higher than that of the pericycle. These investigators explained this curious anomaly by assuming that the suction pressure on the two sides of the endodermal walls was not the same, and that in their experiments the mean of the two values was necessarily taken.

According to Ursprung and Blum (1921), the suction pressure of root cells quickly accommodated itself to the osmotic pressure of the solution to which they were transferred. Bean roots, for example, germinated in sawdust, showed a suction pressure of 1.4 atmospheres, but when they were transferred to a 0.65 per cent. cane sugar solution of osmotic pressure 5.8 atmospheres, the value of the suction pressure rose to 5.7 atmospheres in just over three days. It is therefore possible that the suction pressure of root cells is a measure of the forces of the soil which resist the withdrawal of water, being just greater than these.

Blum (1926) has investigated the suction pressure of alpine plants. Here the aerial regions were always found to give higher values than the root tissues. Seasonal changes, such as rainfall, caused variations in these values. Again, the values obtained for the floral organs were very high. Of the various factors influencing the suction pressure of the cells, soil moisture was found to be the most important. Any decrease in the soil moisture led to an increase in atmospheric humidity, causing strong modifications in the suction pressure. Molz has also shown that soil moisture and atmospheric humidity are important external factors which influence the suction pressure of cells. For example, strong rain after drought may cause a rise of as much as 20 atmospheres in the suction pressure of roots.

Scott and Priestley (1928), and Scott (1928), considered that the

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importance of the root hairs as organs for absorbing water from the soil had in the past been over-emphasised. They also considered that the entry of water into the root must be taken into account in relation to the soil moisture. They supposed that when water is present in the soil in excess and is free to move to the plant, the soil solution permeates the walls of the cortical cells which are composed of cellulose. These cortical cells take up the moisture until they are turgid, apparently acting like a sponge. When the endodermal region is reached, the protoplasts of this layer of cells act as a semi-permeable membrane and the water is drawn in from the outer tangential walls by the osmotic pull of the stelar solution in the inner tangential walls. In these circumstances it is affirmed that the surface area of the root is of no importance. In drier soils, however, in which the soil moisture is not able to move so easily, the root surface and the root hairs are of importance for the absorption of water, and the latter function as water absorbers. This suggestion is of a somewhat revolutionary nature, and it is unfortunate that the authors, before making such statements, did not bring forward strong experimental evidence in support of such a view. It might be suggested that the soil would have to be water-logged to a marked extent before their first assumption would hold, and water-logged soils are not suitable for the normal growth of plants. This work also takes no account of the complex relationship that is known to exist between the soil and its moisture, a relationship that has not yet been clearly elucidated.

### Transpiration

The transpiration of a plant may be defined as the evaporation of water from its aerial parts, such as the stem, twigs and leaves. The amount of water lost by this method is in many cases considerable. Balls has estimated that the loss of water from an Egyptian cotton crop by transpiration is about 50 tons per acre per day or 8 pints per plant.

The main mass of transpiration takes place from the leaves, but it has also been found to occur in the stem as well as in the twigs.

It is well known that transpiration may be divided into two classes: *cuticular* and *stomatal*. Cuticular transpiration is the amount of water lost through the cuticle of a plant; stomatal transpiration is the loss of water through the stomata. In ordinary mesophytic plants stomatal transpiration represents 80 to 97 per cent. of the total water-loss, whereas cuticular transpiration only represents about 2 to 3 per cent. of the total loss. It has been found that even the very thickest cuticles transpire to a certain extent. In some leaves the cuticular and stomatal transpiration is approximately equal. A good example of this is to be found in the Jamaican rain forests, where the cuticular transpiration represents 58 per cent. of the total transpiration of the leaves.

Mature *Citrus* leaves have usually been considered to show greater cuticular than stomatal transpiration. In 1919, Coit and Hodgson reported that from 40 to 50 per cent. of the water loss from mature *Citrus* leaves occurs through the dorsal epidermis and that the stomata become functionless early in the life of the plant. E. T. Bartholomew (1931) has been unable to confirm this statement. He showed that during the day the main water loss (85 to 95 per cent.) from mature leaves is through the stomata. Moreover, the stomata of mature leaves are not functionless, but exhibit the usual diurnal behaviour, opening during the day and closing at night. Similarly, A. R. C. Haas and Halma (1932) have found in *Citrus Limonia*, the so-called eureka lemon, *C. grandis* (marsh grape fruit), and *C. sinensis* (Valencia orange), that transpiration is most rapid from the ventral surface of the leaf on which the stomata are situated. It was also ascertained that the rate of transpiration in these three species is in the order *C. Limonia*, *C. grandis* and *C. sinensis*; *C. Limonia* possessing the highest rate of transpiration and *C. sinensis* the lowest.

In stomatal transpiration the diffusion of water vapour takes place through the open pores of the stomata. The physical side of the matter has been very fully investigated through the now classical investigations of H. T. Brown and Escombe, Renner, Jeffries and others, and will not, therefore, be considered here. Since, however, the main mass of the transpiration of a plant takes place

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through the stomata, it is obvious that external factors play a very considerable part in determining the rate of water-loss. Stomatal diffusion is a process of evaporation. Evaporation from the wet cell walls of the mesophyll cells takes place, and is markedly affected by the external conditions of the atmosphere prevailing at the time. Energy is expended in the process, and it was early shown by H. T. Brown and Escombe that 50 per cent. of the incident sunlight falling on the leaf of *Polygonum Weyrichii* was used in transpiration, and only some 1 per cent. or less utilised for photosynthesis.

### Factors Affecting the Transpiration Rate

The factors affecting transpiration may be divided into two classes : external and internal.

#### *External Factors.*

- (a) Humidity of the atmosphere.
- (b) Wind.
- (c) Temperature.
- (d) Barometric Pressure.
- (e) Light.

#### *Internal Factors.*

- (f) Stomata.
- (g) The water-content of the mesophyll tissue.

**The Humidity of the Atmosphere.** If the atmosphere surrounding the leaf be saturated with water-vapour, no gradient of water concentration can be set up between the intercellular spaces of the leaf and the external atmosphere. In such circumstances no evaporation can take place, and, in consequence, transpiration will be greatly diminished.

The main concern in such a question as this is the water deficit of the atmosphere, and the temperature must be known for a knowledge of this factor. Any rise in temperature will alter the water vapour pressure and cause an increase in the water deficit. Similarly, a decrease in temperature will cause a decrease in the water deficit.

If transpiration be nothing more than a process of evaporation from the wet walls of the mesophyll tissue, there should exist a simple relation between the moisture present in the atmosphere and the transpiration rate.

Francis Darwin (1914) made the first attempt to ascertain, for *Hedera Helix*, the relationship between the transpiration rate and the humidity of the atmosphere when the temperature was kept constant. He discovered that with decrease in humidity there was increase in the transpiration rate, and that a straight line graph was obtained, *i.e.*, there was a simple linear relation between the two. The line, however, when produced backwards, did not

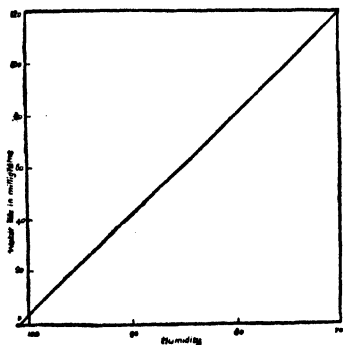


FIG. 10.—Effect of changes of humidity on the transpiration of *Hedera Helix*. (After Henderson, *Anns. Bot.*)

cut the axis at the 100 per cent. saturation point, but at the 105 per cent. saturation point. Since the plant respire in the course of its metabolic activities, the temperature in the immediate vicinity of the mesophyll cells will on this account be higher than that of the surrounding atmosphere. Hence the air in the direct neighbourhood of the mesophyll cells will not be saturated when the external atmosphere is in that condition. It is on this account that the value 105 per cent. was obtained instead of the expected 100 per cent.

From the results of these experiments, Darwin was able to calculate the temperature of the mesophyll compared with the surrounding air. He worked at 16° C. and the vapour pressure

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at this temperature is 13.51. This value, plus 5 per cent., gives a vapour pressure of 14.2. The corresponding temperature to this vapour pressure is 16.8° C. Thus the mesophyll cells were 0.8° C. higher in temperature than the surrounding air.

These observations of Darwin have been in large measure confirmed by Henderson (1926) using more controlled conditions. He found that in *Hedera Helix* his curve (Fig. 10) did not cut the axis as far back as Darwin's, and that the temperature of the mesophyll cells was 0.4° C. and not 0.8° C. higher than the surrounding atmosphere. He was further able to show that the effect of changes of humidity on the rate of transpiration for higher humidity values was to make the surface of the cell act as a damp surface in a purely physical way, since the graphs follow within fairly narrow limits the equation for change in rate of water-loss with change of humidity:—

$$E = E_1 \left( \frac{S_{T_1} - \frac{y}{100} \cdot S_{t_1}}{S_T - \frac{x}{100} \cdot S_t} \right)$$

where  $E$  is the evaporation rate,  $S_T$  is the saturation vapour pressure of air at leaf temperature  $T^\circ$ ,  $\frac{x}{100} \cdot S_t$  is the percentage of saturation water vapour pressure at a temperature  $t^\circ$ ,  $E_1$  is the rate of evaporation when  $S_{T_1}$  is the saturation vapour pressure of the air at leaf temperature  $T_1^\circ$ , and  $\frac{y}{100} \cdot S_{t_1}$  is the percentage of saturation of the water vapour pressure at air temperature  $t_1^\circ$ .

**Wind.** The effect of wind on the transpiration rate is to remove the layers of saturated air or partially saturated air which cover the leaf surface. This will lead to an increase in the transpiration rate.

**Temperature.** In connection with transpiration, temperature has also to be taken into account. Temperature plays no direct part in affecting the water-loss from leaves, but acts in the indirect capacity of affecting the saturation deficit of the atmosphere.

Increase of temperature will lead to an increase of the saturation deficit and thus to an increase in the rate of transpiration.

Humidity, temperature and wind can all be expressed in terms of the evaporating power of the air, and it is a comparatively simple matter to determine the rate at which water is evaporated from a surface.

**Light.** Light materially affects the transpiration rate. When, however, the effect of light is being investigated, it is of the utmost importance that all the other factors involved are kept strictly constant. The matter becomes difficult when investigations are conducted under field conditions in the open. It is therefore more convenient for comparison to use a record of the rate of evaporation from a standard water surface at the same time. This expression summarises the influence of the prevailing atmospheric conditions and makes it an easy matter to detect any differences between the effects of these on transpiration rate and evaporation rate.

Some years ago, Livingston (1906) suggested a method of measuring the evaporating power of the atmosphere, a value which he termed  $E$ . If  $T$  represented the transpiration rate, the ratio  $T/E$ , referred in both cases to unit area, was termed by him the *relative transpiration*. Relative transpiration eliminates the direct evaporating power of the air and the physiological behaviour of the leaf is exhibited.

The main difficulty in this connection is to measure  $E$  in a satisfactory manner. It would, of course, be possible to place an uncovered basin in the open and find the loss of water by weighing at intervals. This method, however, has several disadvantages in actual practice. One of the main sources of error in such a procedure is the fact that the rate of evaporation will alter as the water level falls below the rim of the vessel. Livingston escaped from the difficulty by means of a device known as the "porous cup-atmometer." Various types of these are now in use. A common variety consists of a flask fitted with a cork and glass tube at the other end of which is attached a porous porcelain candle. Flask, tube and porous candle are filled with water, and the evaporation determined by weighing at intervals. Another

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method of comparing T/E is by means of Pische's device. This, in essence, is very simple. It consists of a piece of filter-paper placed over the mouth of a tube containing water. It is not, however, very convenient to use in the field.

The question arises as to how far the atmometer responds in the same way as the leaf. Livingston assumed that by his conception of relative transpiration the direct effect of the atmospheric conditions on the transpiration rate could be neglected in the interpretation of his results. The main assumption underlying this principle is, that changes in the atmospheric environment affect equally the rate of transpiration of a plant and the rate of evaporation from a water surface. As Knight (1917) has pointed out, the assumption is scarcely warranted by the facts. Livingston (1915) and L. J. Briggs and Shantz (1916) have shown that atmometers of different sizes and shapes are not strictly comparable under changing conditions. For example, a change in environment which doubles the rate of evaporation from one atmometer does not necessarily double the rate from another of different size or shape. In exactly the same way the size or shape of the leaf may modify the influence of changes of external conditions on its transpiration rate.

It appears, therefore, that it is not possible to compare one atmometer with another, or one leaf with another, under changing external conditions, so that it seems scarcely justifiable to compare an atmometer with a plant. A further criticism advanced by Knight is to be found in the structure of the leaf. A portion of the path along which the diffusion stream passes from the evaporating mesophyll cells to the outside air is situated actually within the leaf and, being protected by the epidermis, is not, therefore, subject to the direct influence of movements of the outside air. In the case of the atmometer, however, the whole of the diffusion stream is exposed to air movements, and it is theoretically possible by means of a sufficiently rapid current of air to reduce the moisture in the air close to the evaporating surface to the same concentration as that in the general atmosphere. In the case of the leaf, it is only at the surface of the epidermis and not of the evaporating mesophyll that this minimal concentration can be

obtained, so that it is to be expected that changes in the speed of air movement will have less influence on the transpiration rate of a leaf than on the rate of water-loss from an atmometer.

From a large series of experiments, Knight has shown that changes in the velocity of wind affect the plant and atmometer in different ways, and it is only when the air movements are maintained at a constant value that this method of comparison by use of relative transpiration can be used to eliminate changes in relative humidity and temperature on transpiration; since it has been found that these two factors act equally upon transpiration

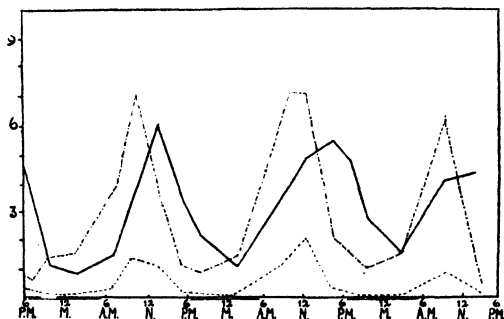


FIG. 11.—Transpiration of *Euphorbia capitellata* through three days compared with evaporation (continuous line) and relative transpiration (dot-dash line). The scale of the ordinates is different in the three graphs. (After Livingston, modified. From Skene, *Biology of Flowering Plants*.)

from a plant and evaporation from an atmometer. It is clear, then, that *only when wind velocity is constant* does relative transpiration give a satisfactory measure of the intrinsic transpiring power of a plant.

A number of years ago Livingston (1906) compared the transpiration of the succulent *Euphorbia capitellata* with the evaporating power of the air, and further ascertained the relative transpiration ( $T/E$ ) at the same time. An examination of his curves (Fig. 11) show a general similarity, but with an important difference. The highest point of transpiration was found to occur between 10 a.m. and 12 noon and this increase was followed by a steady decrease. The evaporation rate, on the other hand, increased between 2 p.m.

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and 4 p.m., at which point the maximum was reached. The prevailing external conditions are evidently of such a nature as to cause maximum evaporation in the late afternoon, whereas some reaction in the plant has led to a diminution of water-loss at an earlier time. The same result is reflected in the graph of relative transpiration. If both the transpiration and the evaporation had been affected in a similar way, this latter curve should have been a

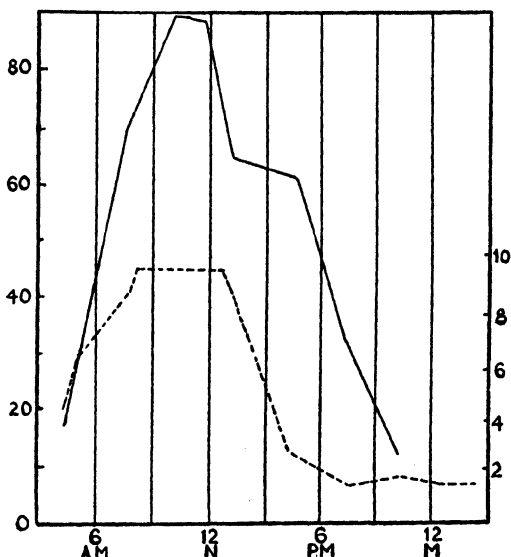


FIG. 12.—Transpiration and stomatal movement. The continuous line represents the transpiration of *Verbena ciliata*, the broken line represents the extent of opening of the stomata in microns. (After Lloyd, modified. From Skene, *Biology of Flowering Plants*.)

straight line. The actual result obtained shows that transpiration is increasing more rapidly than evaporation till the maximum is reached, and the subsequent increase in the evaporating power of the air is not reflected in the transpiration. A further point in this connection is that transpiration increases greatly in light, and decreases in the dark (Fig. 11). The light produces some considerable change in the leaf, and, in actual fact, brings about an alteration in the size of the stomatal openings through which the main mass of transpiration is taking place.

## Stomatal Regulation.

The question as to whether stomatal aperture does or does not control the transpiration rate has led to a good deal of controversy which has only recently been settled. The difficulty here is to employ an accurate technique for measuring stomatal aperture. A variety of methods have been employed by different investigators, but each has its own particular disadvantages. Lloyd stripped off portions of the epidermis of the leaf and placed them

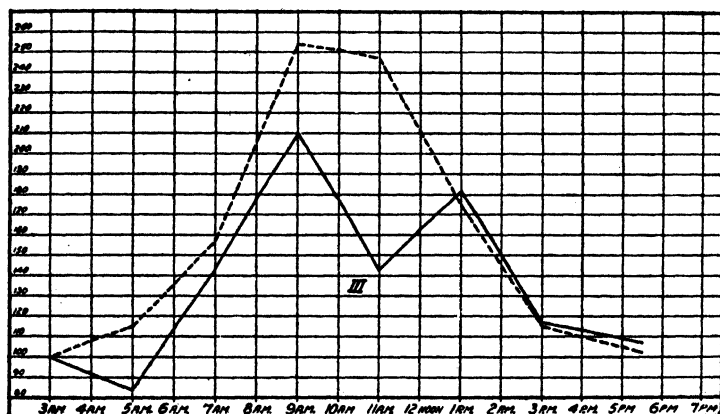


FIG. 18.—Graphs of index of transpiring power of lower leaf surface of *Zebrina pendula*, as determined by hygrometric paper (full line) and average stomatal diffusive capacity as determined by the porometer (broken line). (After Trelease and Livingston, *J. Ecol.*)

in absolute alcohol tinged with Congo red. It was claimed that the walls of the guard cells are rapidly dehydrated and hardened and the stomata undergo no further change in shape. Loftfield (1921) claimed that this is a more accurate method than the porometer, which has been much used in recent years. The porometer method of measuring stomatal aperture was first introduced by Francis Darwin and Pertz, and in this case the size of the stomatal pores is measured by drawing a current of air through them. Knight (1916) found that differences of pressure may cause temporary curvature of the portion of leaf

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under the porometer chamber, and considered that only small differences of pressure should be used, as this curvature may affect stomatal aperture. A further disadvantage of the porometer method lies in the fact that if the current of air be passed through the pores for any prolonged time, the protoplasm of the guard-cells tends to become irritated and as a consequence the stomata close. Ashby (1931) has made a comparison of these two methods and found that they do not give results which differ significantly except at very small stomatal apertures and Lloyd's

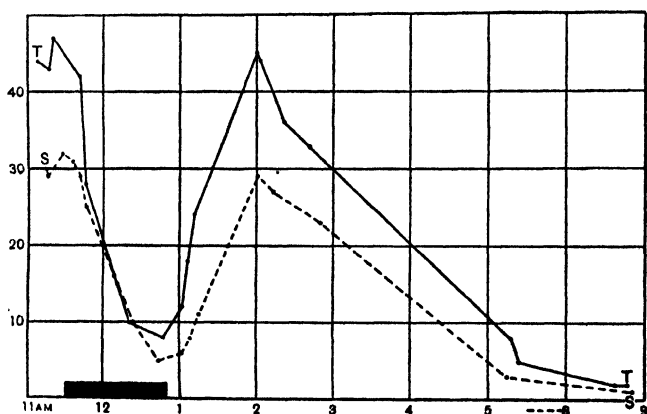


FIG. 14.—Graphs showing relation between stomatal aperture (S) and transpiration (T). (After F. Darwin, *Phil. Trans. Roy. Soc. Lond.*)

method gives the more accurate picture of the diffusive capacity of stomata at small apertures, but very satisfactory agreement was obtained between the two at wide apertures. Lloyd (1908), at the end of his elaborate experiments on the relation between stomatal opening and transpiration rate came to the conclusion that there was no correlation between them (Fig. 12). Trelease and Livingston (1916) arrived at a similar result (Fig. 13) whereas Francis Darwin (1916) from his experimental investigations concluded that a direct relationship existed between the two, and stomatal aperture exerted a direct control on transpiration rate (Fig. 14).

The question now before us is : What is happening in the leaf ? If the stomata were more widely open there should be a greater loss of water ; if the process be one of diffusion, then the gradient should become steeper and altered. External conditions alone will not account for this state of affairs. Within the stomata there is an increase in the water vapour pressure owing to the continued evaporation from the surface of the cells of the mesophyll

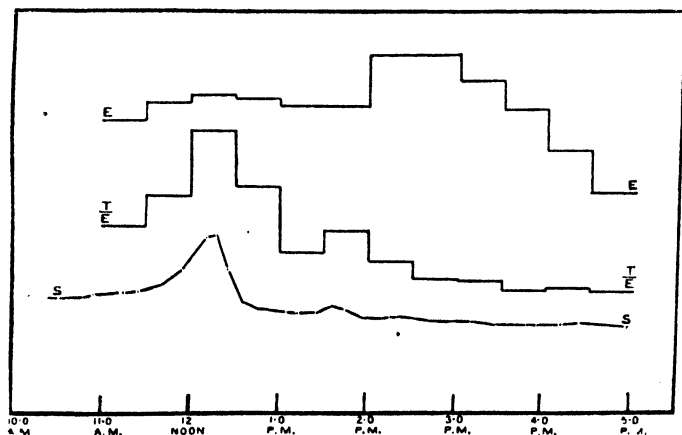


FIG. 15.—Graphs showing parallelism between stomatal aperture and relative transpiration. S = stomatal aperture ; E = atmometer loss ;  $\frac{T}{E}$  = relative transpiration. (After Knight, *Anns. Bot.*)

tissue. There is, in fact, an actual drying of these cells owing to the decreasing water-content of the walls.

The work of Knight (1916, 1917, 1922) on the relation of stomatal aperture to transpiration is fundamental in this connection, and his original papers on the subject should be consulted. This investigator by means of new apparatus and new experimental methods has cleared away many of the old difficulties and cast quite a new light on the whole subject. ✓

Knight first showed that occasionally the graph for stomatal aperture and transpiration rate exhibited a correlation (Fig. 15), whereas, on other occasions, no such connection was found to

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exist (Fig. 16). Employing a special "air-flue" in which the evaporating power of the air could be kept at a constant value by means of an electric fan and with the light intensity and temperature kept constant, he found that stomatal aperture also remained constant. In the course of these experiments Knight used an automatic porometer (Fig. 17) which agitated a mercury surface by bubbles of air with the result that connection was made with a battery and the resulting current drove a magnetic

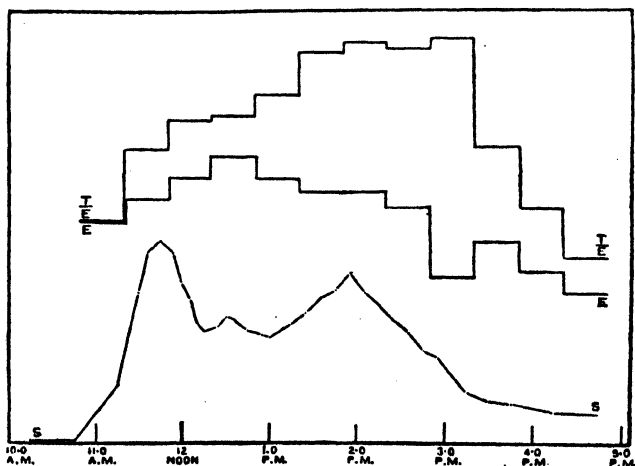


FIG. 16.—Graphs showing lack of agreement between relative transpiration and stomatal aperture. S = stomatal aperture; E = atmometer loss;  $\frac{T}{E}$  = relative transpiration. (After Knight, *Anns. Bot.*)

pen. Having discovered that stomatal aperture and transpiration rate occasionally showed correlation and more often did not, a second series of experiments were initiated which demonstrated that it was the water-content of the mesophyll cells that was the important factor controlling transpiration rate. A cut shoot was placed in water in the air-flue under constant conditions of light intensity and temperature, and the electric fan was started. It was found that the resulting graph for stomatal aperture was a straight line, while the curves for transpiration and evaporating

power of the air also reached a constant value (Fig. 18). The fan was now stopped and the graph for transpiration rate and evaporating power of the air showed an immediate fall, since the layers of aqueous vapour were no longer removed from the leaves.

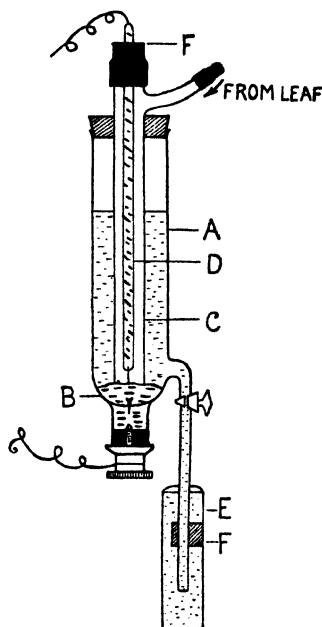


FIG. 17.—Knight's automatic porometer. A = outer glass tube filled with water and with overflow to E. D = glass tube with platinum wire contained in the inner glass tube C, which is attached to the porometer cup on the leaf. B = globule of mercury. Air is drawn through the stomata and intercellular spaces of the leaf through C and depresses the surface of the mercury at B. The air escapes into A and as a result the mercury surface is slightly agitated and makes contact with the platinum point from D, which is first carefully adjusted over B. The momentary current formed drives a magnetic pen. (After Knight, *Ann. Bot.*)

On the other hand the stomatal graph was unaffected. When both transpiration rate and evaporating power of the air had again reached a steady value, the fan was restarted. It was found that while the curve for the evaporating power of the air rose

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to the same value as before, that for the transpiration rate *rose to a higher value* than that previously recorded. The reason for this result is simple. While the transpiration rate was reduced when the fan was stopped, the absorption of water was still going forward in the mesophyll tissue of the leaves at the same rate as before, and water had therefore accumulated in these tissues. When the fan was started once more, the increase in the evaporating power of the air caused an increase in the transpiration rate. In the case of

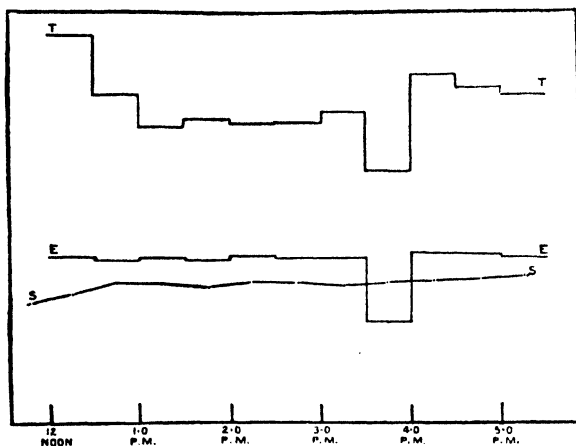


FIG. 18.—Graphs showing an increase in rate of transpiration as a result of increasing water-content of the plant by temporarily decreasing the evaporating power of the air. S = stomatal aperture; E = atmometer loss; T = transpiration. (After Knight, *Anns. Bot.*)

the evaporating power of the atmosphere, no accumulation of water was possible in the atmometer, and so when the fan was restarted the rate of water-loss rose to the same value. This latter experiment of Knight shows very clearly that it is the water-content of the mesophyll tissues and not the opening and closing of the stomata which is the controlling factor of transpiration.

( Knight has also shown that the increase in stomatal aperture which always occurs at the beginning of wilting is invariably accompanied by an increase in the transpiration rate. The

subsequent decline in transpiration is reached, however, before the stomata approach their maximum opening. This decline in the transpiration is due to a fall in the water-content of the mesophyll.

It must be remarked here that in the course of all his experiments Knight failed to take into account the cuticular transpiration of the leaf. It has already been stated that in certain cases this value may be very large—in the Jamaican rain forests appreciably larger than stomatal transpiration—and it is within the bounds of possibility that this cuticular transpiration, small though it be in most cases, may play some important part in the matter.

Loftfield (1921) has made an elaborate study of the behaviour of stomata under a variety of diverse external conditions, and has ascertained that at certain specific apertures they do control the transpiration rate. Lloyd's "stripping" method of measuring stomatal aperture was employed.

Loftfield divided plants into three classes according to their stomatal behaviour :—

1. **Cereals.** Here the stomata remain closed at night, and no opening occurs under normal conditions, favourable or unfavourable. During daylight the stomatal aperture is dependent in duration and degree upon favourable conditions of such environmental factors as evaporation, temperature and water-content.

2. **Thin-leaved Mesophytes.** Under favourable conditions the stomata remain open all day and are shut throughout the night. In Alfalfa the stomata open from two to six hours after daylight, remain open from three to six hours, and then gradually close during a period which is approximately twice as long as that required for opening. Should conditions become less favourable, partial or complete closure may take place during the middle of the day, and if external conditions become very adverse, closure might occur throughout the day. On the other hand, night opening appears when mid-day closure occurs, and increases in degree and extent with it. Lastly, under extreme conditions, the stomata remain closed all day and open all night, the actual degree of opening being dependent on the water-content.

**3. Fleishy-leaved Plants.** This third group, in which certain thin-leaved plants are also included, may be typified by the potato. Here the stomata are continuously and widely open during the whole of the day and night. As the evaporating power of the air increases beyond a certain point, the stomata tend to close. Based upon the time of closure, Loftfield recognised three sub-groups. Potato stomata at first close just after sunset for a time, but as the water-content decreases, this time extends backwards into the afternoon. In cow-beet, under favourable conditions, the stomata are widely open during the day and close very little during the night. The third sub-group is best exemplified by the onion, where the stomata are wide open at night under conditions of high water-content and low evaporation. If water-content alone becomes low, the stomata close at night, but if the evaporation increases instead, the stomata tend to close during the day. Thus, with medium or low water-content, onion stomata react to increased evaporation as in the Alfalfa group, showing increasing mid-day closure correlated with increased night opening.

In his studies upon transpiration and stomatal aperture, Loftfield found that in certain cases there was a direct connection between the two, while in some cases no such correlation could be discovered. He therefore concluded that stomatal movement has an important influence on the transpiration rate: "When the stomata are widely open or nearly widely open, transpiration is the result of the action of factors of evaporation alone, since the stomata in nowise interfere with the action. As the stomata close, the influence of the factors is lessened, but until closure has reduced the apertures to 50 per cent. or less, stomatal regulation is largely overshadowed by the control exerted by them. When closure is almost complete, the regulation of water-loss by the stomata is very close, and the factors overshadowed by the effect of even very small changes of the opening" (Loftfield).

Stomata, then, do exercise a control over transpiration when closed to a considerable extent, but when they are widely open, they possess no control over the water-loss of the leaf. In such circumstances it is the water-content of the mesophyll which is

the important factor. The controlling influence of the stomata on transpiration must be due to the fact that they are sensitive to the action of light, and not to small changes in the water-content of the leaf.

### The Mechanism of Stomatal Movement

It will be convenient at this stage to consider briefly the mechanism of stomatal movement. The early observations of von Mohl showed that stomata open in the light and close in the dark, and that the opening and closing depend on changes of turgor in the guard-cells. Von Mohl observed the presence of chloroplasts in the guard-cells, and considered that their turgor was raised by the photosynthetic activity of the plastids in light with the formation of soluble sugars and coincident increase of osmotic pressure. It was shown by Leitgeb, however, that the stomata open in the light in the absence of carbon dioxide, while the later experiments of Lloyd (1908), Iljin (1914, 1922), and Loftfield (1921) showed that the opening in light is accompanied by a decrease in the starch-content of the guard-cells, and at closure the starch-content is increased. In other words, the behaviour of the guard-cells was the direct opposite to that of the assimilating cells of the mesophyll. Such being the case, the decrease in the starch-content must lead to a considerable increase in the osmotic pressure of the guard-cells giving the necessary conditions for stomatal opening. Wiggans (1921), in an important publication, ascertained the osmotic pressure of the guard-cells and the epidermal cells at different times during the day. With *Cyclamen* the osmotic pressure rose from 14.6 atmospheres at 7 a.m., to a maximum of 31.0 atmospheres at 11 a.m., and then gradually fell to 18.5 atmospheres at 5 p.m. The epidermal cells, on the other hand, remained at a constant value of 10.2 atmospheres throughout. Similarly, in the beet, the epidermal cells showed a constant osmotic pressure of 12.5 atmospheres, while the osmotic pressure of the guard-cells rose from 23.5 atmospheres at 7 a.m. to 31.6 atmospheres at 11 a.m. It remained constant at this value till 1 p.m., and then gradually

fell to 25·0 atmospheres at 5 p.m. Similar results were obtained by Sayre (1923) for *Rumex Patientia*. The guard-cells at night when the pore was closed showed an osmotic pressure of 13 to 14 atmospheres; when fully open at noon a value of 23 atmospheres was recorded. The subsidiary cells showed a constant value of about 15 atmospheres, and the other epidermal cells about 13 atmospheres.

Lloyd, Loftfield and others, have supposed that the change of starch into sugar and sugar into starch is in the nature of a reversible enzymic reaction which is in some way influenced by light. Both Lloyd and Loftfield, for example, found that under screens of blue glass, stomata open and hydrolysis of starch into sugar takes place in the guard-cells and it is a well-known fact that blue light considerably lowers the rate of assimilation. A paper by Baly and Semmens (1924) bears on this point. It was claimed by these workers that polarised light has a very material effect in accelerating the hydrolytic activity of diastase on starch, and it is possible that since normal daylight contains a considerable amount of polarised light this may have some influence on stomatal opening. On the other hand, Neilson Jones (1925) stated that polarised light has no such action on diastase, and although it is no doubt true that the technique employed by Baly and Semmens left a very great deal to be desired and was certainly open to the justifiable criticisms made by Neilson Jones, this work should be repeated under still more critical conditions, for if this work of Baly and Semmens be correct it explains a good deal that is at present obscure (see Chap. VIII). The question arises as to how far does the influence of light affect the direction of the equilibrium. Leitgeb, F. Darwin, and Lloyd have shown that a reduction in the partial pressure of the carbon dioxide surrounding the leaf led to the opening of the stomata, while Linsbauer (1917, 1926) discovered that increase in the partial pressure of the gas led to the opposite result. Weber (1923, 1926a, 1926b, 1927a, 1927b) connected these facts with the action of the light, and assumed that the concentration of carbon dioxide in the guard-cells was regulated by the rate of its photosynthetic absorption. Both Linsbauer and Weber have shown that remarkable physiological

reactions accompany the functioning stomata. The plastids changed their position, the nucleus underwent changes in size and shape and the open and closed stomata showed differences in their power of reducing silver nitrate.

More recently Sayre (1923) and Scarth (1926, 1927) have shown that these various changes observed in the guard-cells are secondary phenomena of a simple common case. Using solutions which readily penetrated the guard-cells, such as solutions of ammonia and acetic acid, Scarth found that the stomata opened in both alkali and acid, but more fully in the former. In an intermediate range of  $pH$  (5.5 to 7.0) for *Zebrina pendula*, the stomata remained closed, but opened in increasing concentrations of both alkali and acid up to the limits of injury. It was further discovered that starch made its appearance in the  $pH$  zone of closure and disappeared in that of opening, and these changes were reversible. Scarth also ascertained that the sap of the guard-cells under normal conditions possessed a higher  $pH$  (i.e., were on the alkaline side) when the stomata were open, and that a low  $pH$  was registered when they were closed. The range appeared to stretch from  $pH$  7 to 4.5.

Sayre obtained very similar results for *Rumex Patientia*. The guard-cells showed a more alkaline reaction when the stomata were open, and the movement could be controlled at will by variations in the  $pH$  of the guard-cells. The stomata opened within the range of  $pH$  4.2 to 4.4 and closed on both sides of this. Sayre considered that there was an optimum  $pH$  for the enzymic hydrolysis of starch, whereas Scarth thought from the fact that there are two widely separated optima, and that the direction and rate of the reaction appear to have no relation to the relative concentrations of the reagents, that stomatal opening is not explicable on a simple enzymic basis.

The metabolism of the stomata of white-margined *Pelargonium* leaves has been investigated by Kümmler (1922), who showed that they contained abundant starch—more, in fact, than the guard-cells of normal green leaves—and that they opened in light and closed in the dark. Very wide opening only occurred if the water supply were highly favourable. These results appear to

show that chlorophyll is not an essential part of the plastids of the guard-cells, and it is possible that they are in the nature of leucoplasts.

External conditions, such as humidity, temperature, etc., all play a part in influencing stomatal opening. Loftfield (1921) has shown that temperature markedly affects stomatal movement and that for a rise of  $10^{\circ}\text{C.}$  the rate of movement is approximately

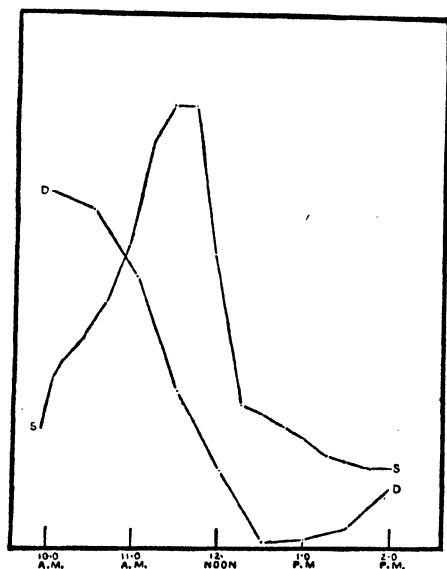


FIG. 19.—Graphs showing the continued opening of stomata in presence of increasing water-loss. S = stomatal aperture; D = water deficit. (After Knight, *Annals Bot.*)

doubled. Excessive transpiration may lead to a loss of turgor by all the cells of the leaf, including the guard-cells, when collapse and closure of the stomata takes place.

The investigations of Knight (1917) have largely cleared away the conflicting statements that have been made from time to time concerning the effect of wilting on stomatal behaviour. F. Darwin (1898) considered that the stomata opened widely in the early stages of wilting, and Knight has confirmed this observation.

When the leaf is losing water more rapidly than water is supplied to it, there is a temporary increase in stomatal opening (Fig. 19). Only when the wilting is very pronounced does closure take place.

The complexity of the stomatal mechanism is shown by the recent work of Maskell (1928), who discovered a well-marked rhythm in the stomata of *Prunus Laurocerasus*. Under constant artificial light intensity the stomata began to close towards the evening and to open about midnight (for a full description of these experiments, see Chapter II).

It must be confessed that our knowledge of the underlying mechanism which controls and regulates stomatal behaviour still leaves much to be discovered.

### **The Effect of Light on Transpiration from the Mesophyll**

Although a great deal has been done in the way of discovering the effect of stomata on the rate of transpiration and also a number of facts and figures are available for the transpiration of the leaf as a whole, very few attempts have been made to ascertain the direct effect of changing external conditions on the mesophyll alone, although it is from these cells that the main water loss occurs. The action of the mesophyll has in most cases never been separated from that of the stomata. The difficulty is to remove the action of the stomata. It was suggested by Knight that leaves from which the epidermis had been stripped might be employed, but the method is drastic, and there is the further danger of the drying of the mesophyll cells except in atmospheres of high humidity.

The other method was that employed by F. Darwin (1914). The essentials of this method are to exclude the action of the stomata by stopping them up with some substance like vaseline and then placing the intercellular spaces in contact with the atmosphere by means of slits in the leaf tissue. Such slits were usually made with a scalpel between the principal veins of the leaves so as to interfere as little as possible with the water supply. Using this method, Darwin attempted to determine the effects of changes of light and humidity of the atmosphere on the mesophyll tissues of

the leaf. He came to the conclusion that the leaf increased its transpiration by amounts that varied between 10 to 100 per cent. of that in the dark. An increase of the latter order is certainly most remarkably high. To bring about such a doubling of the evaporation rate from a moist surface in air of constant evaporating power would require an increase of temperature of at least 10° C. From a careful consideration of Darwin's data, Henderson (1926) was able to ascertain two possible sources of error. Firstly, Darwin's observations were usually begun immediately after smearing the leaves with vaseline and slitting, thus making no allowance for "wound-shock response," and secondly, external conditions were seemingly quite uncontrolled and largely unknown. The plants were in some cases removed from the window of the laboratory into the dark-room, apparently without accurate observations being made of humidity and temperature.

Henderson, by means of a highly ingenious piece of apparatus, has repeated and extended Darwin's observations. The plants used in these experiments were *Hedera Helix* (Darwin's original plant), *Eupatorium adenophorum* and *Aster* (sp.). It was found that the transpiration at first rose to a high value after slitting, and then settled down to a steady rate in the course of an hour or so. The cause of this strange behaviour was not ascertained. It was during this preliminary unsettled period that all Darwin's readings were taken. Since all his experiments were begun in the light and the plants removed to the dark-room when the maximum was reached, it was not surprising that a drop was shown in the transpiration rate in the dark, and it is also of significance in this connection that when the plants were again placed in the light, the second curve nowhere reached the same value as the first. Had Darwin started his experiments in the dark, then in all probability his results would have been reversed.

It was shown by Henderson at the conclusion of his experimental work that light does have a small direct effect upon transpiration from the mesophyll; the order of increase being from 4 to 5 per cent. of that in the dark. The exact action of the light on the mesophyll is unknown. Henderson has suggested that perhaps the light reduces the resistance of the protoplasm

of the mesophyll cells to the passage of water. If such be the case the supply available to the cell walls will be increased, their imbibition will be higher and the vapour tension at the evaporating surface of the walls will tend to rise. In this manner equilibrium at a more rapid rate of water loss will be established.

In any case this action of the light shows that transpiration is not solely a simple process of evaporation. It is possible that a secretion of water from the mesophyll cells of the leaf takes place, the cells actively secreting water or some solution on their outer surfaces. Here again, it must be confessed that the word "secretion" in reality only acts as a cloak for our ignorance of the chemical and physical phenomena underlying the whole process.

### **Transpiration of Xerophytes**

The recent publication of an English translation, under the editorship of the late Professor Yapp, of Professor N. A. Maximov's work, "The Plant in Relation to Water," has necessitated a consideration of the whole question of the transpiration of xerophytes anew. It was a most unfortunate fact that the translation appeared too late to include Maximov's ideas in the first edition of this book.

The term xerophyte is usually given to plants inhabiting regions in which the water supply is deficient. Plants living under such conditions usually possess a number of structural peculiarities by means of which they are able to protect themselves against drought.

Taken as a group, xerophytes may be subdivided into a number of different classes, depending on the method employed to overcome deficiencies in the external water supply.

Reduction of transpiration has always been considered a feature of the whole class of xerophytes. This is certainly the case for succulents of the Cactus type. Such plants possess a massive parenchymatous tissue capable of storing considerable amounts of water and a thick and heavy cuticular covering which prevents a high rate of transpiration. Succulents form the characteristic vegetation of the New World deserts, and the semi-deserts of South Africa, but their occurrence is rare in the deserts

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of the Old World. Other characteristics of such succulents are low osmotic pressure and a small and superficial root system. Their somatic organisation makes them admirably suited for the slow loss of water. Maximov quotes Burgerstein on the comparison of loss of water from the stem of *Opuntia* and the leaf of *Hydrangea*, a typical mesophyte. The ratio here was found to be approximately 1 : 32. Similarly Maximov also gives the early work of MacDougal and Spalding (1910) which bears on this point. These investigators ascertained the amount of water lost from a species of *Echinocactus* over a number of years. The original plant weighed 37.8 kg. and was left unwatered in the laboratory for six years and weighed at intervals. In the first year it lost 3,443 gm., in the second 2,080 gm., 1,535 gm. in the third, 1,400 gm. in the fourth, 1,165 gm. in the fifth, and in the sixth year 1,280 gm. It is thus evident that succulents transpire but slowly and are able to withstand prolonged periods of drought.

Other peculiarities of succulents lie in their respiration and assimilation. In succulents respiration ends frequently at the organic acid stage (see Chapter VI), and the organic acids formed in this way later provide a supply of carbon dioxide for assimilation. Maximov therefore claimed that the physiological peculiarities of these plants make them more akin to epiphytes which are dependent on water absorbed during rains, than to those of true xerophytes.

A further group of plants which are classed among xerophytes possess a variety of structural modifications to prevent excessive transpiration. The presence of sunken stomata is one of the best known of these modifications. *Hakea pectinata*, *Agave americana* and *Dasyliirion filifolium* serve as good examples of this type of xerophytic vegetation. The interposition of a sunken chamber between the diffusing stomata and the external air allows of the former attaining a high degree of humidity with the result that transpiration is materially reduced. Renner (1909) has calculated that the presence of such pits leads to a fall in transpiration of 81 per cent. in *Agave americana*, while the more complex structure found in *Hakea pectinata* leads to a fall of 87 per cent. The double system of chambers in *Dasyliirion filifolium* will cause a still

further depression in the transpiration rate. The presence of the stomata themselves at the base of these pits or chambers is especially important in reducing the effect of wind on the rate of transpiration.

The ability of leaves to roll themselves into a tube with the stomata on the inner surface is yet another method of obviating excessive transpiration. The grasses *Psamma* and *Stipa* are examples of this type of xerophyte.

It is, however, with the so-called *sclerophylls* of ecologists that Maximov's work is mainly concerned. Sclerophylls, as a class, are characterised by a thick cuticle on the upper surface of their leaves and an abundance of supporting mechanical tissue. This type of xerophyte has a wide distribution in the desert regions of the Old and New World and along the coast of the Mediterranean seaboard. In the regions bordering on the Mediterranean the vegetation is subjected to two periods of drought annually; firstly, in the summer months, and secondly, in the somewhat mild winter. In the latter period transpiration exceeds water absorption owing to the cooling of the soil.

It has been shown by Maximov and his co-workers that sclerophylls, unlike succulents and plants with sunken stomata or rolled leaves, possess a high intensity of transpiration. This view is quite contrary to orthodox belief, for it was never doubted until this work that sclerophylls possessed a reduced transpiration rate.

The term *intensity of transpiration* is used by Maximov in the same sense as Ivanov (1913), who defined it as the quantity of water lost by a plant in unit time, per unit of transpiring surface (usually the leaves). In 1920, Burgerstein made the suggestion that the hour be taken as the unit of time and the square decimetre as the unit of transpiring surface and the gram as the unit of weight of water transpired.

Using the term intensity of transpiration in the sense defined above, Maximov carried out comparative experiments on a number of different types of xerophytes and mesophytes. The rapidity with which water stored in the leaves was expended was also ascertained at the same time. This was calculated as percentages of the water-content of the transpiring surface per hour and was

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used to indicate the rate of change of the water conditions of the leaf. The experimental work was conducted at Tiflis, in Russia, where climate and vegetation are of the semi-desert type. The results are summarised in Table I:—

TABLE I

*Intensity of Transpiration and Rapidity of Expenditure of Water by Xerophytes and Mesophytes (according to Maximov, Badrieva and Simonova). (From Maximov, "The Plant in Relation to Water," Eng. Edit., p. 269.)*

Species.	Intensity of Transpiration.	Rapidity of Expenditure of Water Store.	Character of the Leaves.
<b>A. XEROPHYTES</b>			
<i>Sedum maximum</i> . .	2·8	8	Succulent.
<i>Zygophyllum Fabago</i> . .	4·9	15	Semi-succulent.
<i>Gypsophila acutifolia</i> . .	5·4	20	Hard, fleshy.
<i>Caccinia Rauwolfi</i> . .	8·8	44	Hard, encrusted with lime.
<i>Verbascum ovalifolium</i> . .	8·8	71	Densely hairy.
<i>Glaucium luteum</i> . .	9·2	40	Fleshy, covered with wax.
<i>Salvia verticillata</i> . .	9·9	55	Hard, fleshy.
<i>Stachys Kotschy</i> . .	12·7	119	Densely hairy.
<i>Cladochæta candidissima</i> (= <i>Helichrysum candidissimum</i> )	13·2	40	Densely hairy.
<i>Falcaria Rivini</i> (= <i>F. vulgaris</i> )	13·7	87	Hard, covered with wax.
<b>B. MESOPHYTES</b>			
<i>Lamium album</i> . .	3·6	58	Shade plant.
<i>Viola odorata</i> . .	4·0	58	Shade plant.
<i>Vinca major</i> . .	4·5	45	Shade plant with leathery leaves.
<i>Campanula rapunculoides</i> . .	4·8	36	Shade plant.
<i>Sisymbrium Loeselii</i> . .	8·3	62	Sun plants of the spring vegetation.
<i>Hirschfeldia adpressa</i> . .	9·8	40	
<i>Erodium ciconium</i> . .	9·2	83	

The figures given in the table only refer to conditions under which water lost by transpiration from the leaf surface is replaced

by absorption. If the expenditure from transpiration were not replaced, the leaves would wilt and this would inevitably lead to an immediate decrease in water loss.

It is evident from the figures given by Maximov in this table that in general the xerophytes used<sup>1,2</sup> possess a higher intensity of transpiration than the mesophytes. The only exceptions that were \* discovered were *Sedum maximum*, a succulent, and the two semi-succulents, *Zygophyllum Fabago* and *Gypsophila acutifolia*. Among xerophytes with a high intensity of transpiration are *Verbascum ovalifolium*, *Stachys Kotschyi* and *Helichrysum candidissimum*, whose leaves are densely covered with hairs, while *Glaucium luteum* and *Falcaria Rivini* possess a thick cuticle with a deposit of wax. Such data as are presented here appear to confirm the suggestion that these various means of protection have little significance during normal transpiration and are only of value during wilting.

The case of *Encelia farinosa*, an inhabitant of the Colorado Desert, which has been investigated by Shreve (1920), is interesting in this connection. It was ascertained that this plant possesses the power of cutting down its relative transpiration during the months in which aridity is increasing. The plant possesses two distinct types of leaves: a mesophytic type present during the cool months of the year, and which is shed when arid conditions approach, and in turn is succeeded by a xerophytic form. It was discovered that when discs of uniform section were cut from the two types of leaves, those obtained from the xerophytic form lost water 1.44 times more rapidly than discs cut from the mesophytic form; although, if the water loss were calculated on a dry-weight basis, the discs from the xerophytic leaves only lost water 0.78 times as fast as the corresponding mesophytic discs. On the other hand, Shreve discovered that while arid conditions were in existence the xerophytic leaves secrete a brown, viscous fluid which appears to play a considerable part in hindering excess of transpiration.

An examination of the transpiratory organs of *Larrea tridentata* (*Covillea tridentata*, creosote bush) has been effected by Ashby (1932). *L. tridentata*, which colonises the driest areas around

Tucson, Arizona, is a perennial with small, sticky, resinous leaves, oppositely arranged along a much-branched stem. Ashby compared *L. tridentata* with a mesophyte, *Ligustrum* (sp.), for the following points: (a) Ratio of leaf surface to total surface of aerial organs, (b) frequency and distribution of stomata, (c) anatomy of stomata, (d) rate of drying out of cut twigs, (e) water-content of leaves, (f) suction pressure of roots, and (g) conductivity of stems for water.

It was shown that *Larrea tridentata*, far from possessing a reduced leaf area, has in point of fact a greater leaf area than *Ligustrum*. The stomatal area was also found to be bigger in *Larrea*. The stomata showed no anatomical peculiarities such as are found in *Dasyllirion* and *Hakea*. With regard to water loss it was found that *Larrea* is much less economical than *Ligustrum* under conditions of drought and is capable of losing as much water in a day as a privet bush of comparable size, provided that the stomata remain open. The suction pressure of the roots was also found to be greater, 25 atmospheres for *Larrea* and 7 atmospheres for *Ligustrum*. Comparison of the resistance to the passage of water under pressure in a mature stem of *Larrea* shows that it has twice the resistance offered by a stem of *Ligustrum* of the same length and area of woody tissue.

The evidence brought forward in this paper strongly supports Maximov's suggestions, that low transpiration *qua* low transpiration is not a characteristic of xerophytes and that their power of drought resistance must be sought elsewhere.

According to Maximov, xerophytes, other than succulents and plants possessing various structural modifications to reduce transpiration, *e.g.*, sunken stomata, rolled leaves, etc., are characterised anatomically by decrease in the size of the cells of the leaf, including the stomata, a thickening of cell walls, strong mechanical development of palissade tissue, a denser network of veins and an increase of stomata per unit area of leaf. On the physiological side, he concluded that xerophytes show a high osmotic pressure, a high intensity of transpiration and assimilation, and the capacity of enduring prolonged conditions of wilting. They are able to endure prolonged shortage of water supply, not

by reducing their intensity of transpiration, but in the property of their protoplasm to withstand desiccation. Ordinary mesophytes quickly show wilting by flagging of their leaves, and in this condition the leaves are soon torn and injured by wind. Sclerophylls, on the other hand, with their tough cuticle and abundance of mechanical elements, are enabled to pass through such conditions unharmed and give no external indication that in time of drought they are in a wilted state.

In connection with the high osmotic pressure and decreased size of the cells of xerophytic leaves, Walter (1926) has advanced a very ingenious hypothesis. He pointed out that in the leaf there is a physiological equilibrium between starch and sugar, *i.e.*,  $\text{Starch} \rightleftharpoons \text{Sugar}$ , which depends upon the water-content. When there is an adequate water-content, starch is condensed from sugar; whereas, should the turgor fall, hydrolysis of starch will set in and the equilibrium will be the direction of increased sugar formation. Increase in the concentration of sugar will raise the osmotic pressure. In the cell there is a condition of equilibrium between the water held osmotically in the cell sap and that held by imbibition in the protoplasm. Any increase in the osmotic pressure in the cell sap will result in the removal of water held by imbibition in the protoplasm. Walter suggested that the normal, full cell size is not attained when the protoplasm is partially desiccated in this manner; hence the smaller cells, more numerous stomata and increased transpiration of xerophytic leaves.

To return to the example of *Larrea*; in the hot, dry season it becomes quite inactive, whereas the mesophyte *Ligustrum* under similar conditions wilts and does not recover, while *Larrea* recovers at the first adequate shower of rain. It is on this power of recovering from wilting that the success of the sclerophyll in overcoming shortage of water supply depends and not on reduced intensity of transpiration. "That xerophytes should show such a high intensity of transpiration is not really surprising . . . the enormously developed root system of many xerophytes has to supply with water only a comparatively insignificant aerial portion of the plant. Under such conditions a high rate of transpiration is

not only not dangerous, but is rather an advantage, for an energetic gaseous exchange contributes to a more intense assimilation. Further, xerophytes forming the sparse vegetation of open, strongly insolated habitats, belong to the type of 'sun' plants, and the more light such plants obtain, the higher is their transpiration capacity" (Maximov, p. 270). As Yapp has pointed out in a footnote to this passage, there is no direct connection between rate of transpiration and rate of photosynthesis, such as might be inferred from the text. On the other hand, when water balance is such as to lead to the stomata being widely open, rapid transpiration would tend to be accompanied by vigorous photosynthesis.

### **The Importance of Transpiration to the Plant**

Transpiration exists in all land plants. Whether it be an advantage or a disadvantage, the fact remains that it is quite unavoidable. The absorption of carbon dioxide from the air by the leaf is a prime necessity for the synthesis of carbohydrates by the living plant, and it follows that there must be some suitable mechanism of gaseous exchange in the organisation of the plant body, and this exchange is principally performed by the stomata of the leaf. As a corollary, it follows that if stomata are present, water losses must occur. The aerial portion of the plant soma is covered with cuticle, and were this cuticle to extend over the whole surface of the plant, it would be unable to carry out its normal function of carbon dioxide absorption and, of necessity, its metabolic activities would cease.

A large number of teleological explanations have been advanced which were considered as proof that transpiration is vital to the well-being of the plant. The best that can be said for these is that they are explanations. It has been supposed that in plants exposed to extreme conditions of heat, transpiration cuts down excess of temperature. This is possibly true within limits. It certainly does not apply to succulents which transpire but slowly and possess a number of somatic modifications to prevent excessive transpiration. It has also been shown in a number of succulents that the temperature of the mesophyll tissues is very much higher than that of the surrounding air.

In the case of ordinary mesophytes the temperature of the leaves is not much higher than that of the surrounding atmosphere, usually about a degree or so. In high winds the temperature may even fall below that of the air. Even when leaves are cut the temperature only rises one or two degrees from "wound-shock." It must also be remembered that in the case of the ordinary type of dorsiventral leaf with its flat surface, there is a very large area exposed to the air which would tend to prevent any rise in temperature should external conditions of drought and heat arise.

Miller and Saunders (1923) have very fully investigated the relationship between the rate of transpiration and leaf temperatures under conditions of limited water supply. In various crop plants (pumpkin, corn, sorghum, cowpea and others) they determined the leaf temperatures by means of a thermo-couple method when the leaves were turgid and transpiring heavily, and also in wilted leaves in which transpiration was reduced. In the cowpea it was found in one instance that the air temperature was  $37.0^{\circ}\text{C}$ ., while in the turgid leaf it was  $36.5^{\circ}\text{C}$ . and in the wilted leaf  $46.0^{\circ}\text{C}$ . and the maximum difference between the rates of transpiration of turgid and wilted leaves respectively was 20 : 1. A number of determinations were made between 9 a.m. and 4 p.m., and it was shown that on the average the ratio of the average rate of transpiration of turgid leaves to that of wilted leaves was 3.5 : 1 in soya beans and cowpeas, and the ratio was 2.5 : 1 for sorghum and corn. The temperature of turgid leaves was on the whole but little different from the surrounding air temperature. These results appear to indicate that transpiration may play some part in reducing the temperature of the leaf.

The most exact work on this subject has been carried out by Clum (1926) using leaves of *Fuchsia speciosa*, *Brassica oleracea* and *Syringa vulgaris*. The temperature of the leaves was determined by means of thermo-couples under a number of experimental conditions. The leaves were always found to be at a higher temperature than that of the surrounding air. The effect of shading was to produce a sudden drop in the temperature; further, the direct effect of sunlight was very much marked, and the particular angle at which the rays struck the leaf had an

effect on its temperature. In no case, however, could he find any correlation between leaf temperature and transpiration.

A second claim has been put forward to show that transpiration is an advantage to the plant in that it rids it of excess water and allows of the rapid absorption of salts. A considerable amount of evidence was at one time forthcoming in support of this view, but it has since been shown to be erroneous. The process of transpiration has nothing to do with the absorption of salts; the two processes work independently of one another. The absorption of salts is a question of the salt and water equilibrium of the cell, and is not connected with the evaporation of water from the leaves. Moreover, the concentration of salts in the plant is very much higher than the concentration of the corresponding salts in the soil. An early experiment of Hasselbring (1914) bears on this problem. He grew tobacco seedlings in the open where transpiration would be normal, and a second series under calico to reduce transpiration. At the end of a given time the ash-content of the two sets was determined, and was found to be identical in both series. The ash of the shade series was 11.2 per cent., compared with 9.2 per cent. for those grown in the open. The shade plants had absorbed 35 litres of water and the sun plants 46 litres or 30 per cent. more. The amount of mineral matter absorbed by the plants was therefore not proportional to the transpiration, nor did the plants with the lower transpiration suffer with regard to salt absorption. Later experiments by other investigators have given substantially the same results. Muenschner (1922) grew barley in water culture, and, by suitable arrangements, was able to cut down the transpiration rate. He ascertained that there was no difference in the ash-content of the series grown under conditions which reduced transpiration; the ash-content could only be correlated with the growth of the plants. Mendiola (1922) found that in tobacco a reduction in the amount of transpiration led to an increase in the dry-weight and a decrease in the ash-content. On the other hand, his results support the view that there is no proportionality ? between the amount of transpiration and the amount of salt absorbed by the plant.

It is quite possible, however, that transpiration might increase the rate of transport of salts from one part of the plant to another, once the salts had effected an entrance. The rate of the transpiration stream is rapid, while the passage of salts from cell to cell is slow. It has been shown that salts are present in the xylem, and it may well be that the transpiration stream conveys salts from the lower to the upper regions of the plant. There may be a critical rate of the transpiration stream below which the water fails to carry the salts in the xylem with any rapidity.

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## CHAPTER II

### CARBON ASSIMILATION

*Theory of Limiting Factors—The First Sugar of Photosynthesis—Chemical Mechanism of Photosynthesis—Chemistry of Chlorophyll—Photosynthesis and Chlorophyll Content—Chloroplasts and their origin.*

THE whole subject of carbon assimilation has been so recently reviewed in two extensive monographs by Stiles (1925), and Spoehr (1926), that only a few important aspects fall to be recorded here.

One such aspect is the question of "limiting factors" which still gives rise to active discussion. In 1905 F. F. Blackman, in his classic paper on optima and limiting factors, described a scheme to account for the interaction of a number of factors in their effect on the assimilation process which is best stated in the author's own words: "When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the 'slowest' factor." That is to say, the factor present in lowest concentration limits the rate of the process. To discover which is the limiting factor the following principle is applied: "When the magnitude of a function is limited by one of a set of possible factors, increase of that factor, and that factor alone, will be found to bring about an increase of the magnitude of the function."

The graph demanded by the Blackman theory was a straight line showing a sharp break when the limiting factor came into operation (Fig. 20). Such graphs were obtained by Matthaei (1904), Blackman and A. M. Smith (1911), and Wilmott (1921), and the validity of the theory was accepted without question for over a decade.

Hooker (1917) and W. H. Brown (1918) both submitted the theory to a good deal of destructive criticism. Brown considered

that the curve obtained by Blackman and Smith for the assimilation rate of *Elodea* at two different light intensities was in reality

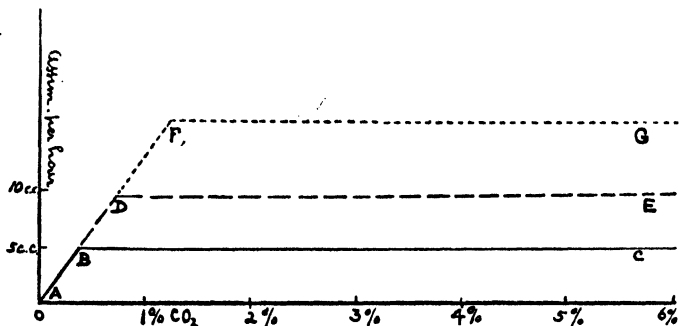


FIG. 20.—Curves illustrating the theory of "limiting factors." (After F. F. Blackman, *Ann. Bot.*)

made up of two separate curves, and that no sharp break existed. There was therefore no operation of limiting factors. The whole

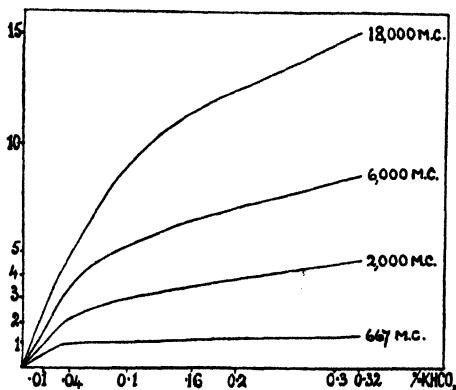


FIG. 21.—The rate of assimilation in different concentrations of potassium bicarbonate and constant light intensity. (After Harder.)

question revolves round this fact: Is there or is there not a sharp break in the curve, or is the curve smooth in form?

Boysen-Jensen (1918), using *Sinapis alba*, found a smooth curve

to exist, and no sharp break was obtained when one of the factors became limiting in the Blackman sense.

The most important investigations on this subject were conducted by Harder (1921, 1923). The material used was *Fontinalis antipyretica*, *Cinclidotus aquatilis* and two species of *Cladophora*. The plants were allowed to assimilate in solutions of potassium bicarbonate of different concentrations, and the rates of assimilation were measured by estimations of the amounts of oxygen evolved by a volumetric method. Harder was able to show that the curves obtained in all cases were smooth in shape and

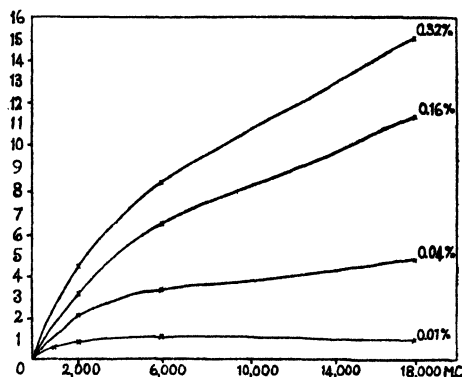


FIG. 22.—The rate of assimilation at different light intensities and constant amounts of potassium bicarbonate. (After Harder.)

exhibited no sharp break. The matter was tested in two ways: (1) all the factors save one were kept constant; and (2) variations in two factors were followed. The curves obtained are shown in Figs. 21 and 22. Harder pointed out that in the course of these investigations the same material was always used, whereas Blackman employed fresh lots for each experiment, and the points of his curves lie on such an irregular line that they might be either continuous or show a sharp break. A consideration of the second series of experiments conducted by Harder, in which two factors were varied together, show that an increase in either factor brings about an increase in the assimilation rate. It is obvious that the factors are mutually interdependent and that the

relationship between the two is of a complex nature, and according to Harder, it is the factor which is in relative minimum which is most important.

Warburg (1919, 1920), using a modification of the Haldane-Barcroft method of gas analysis and the unicellular alga, *Chlorella*, obtained curves similar to those of Harder. He ascertained that in low concentrations of carbon dioxide the assimilation rate was

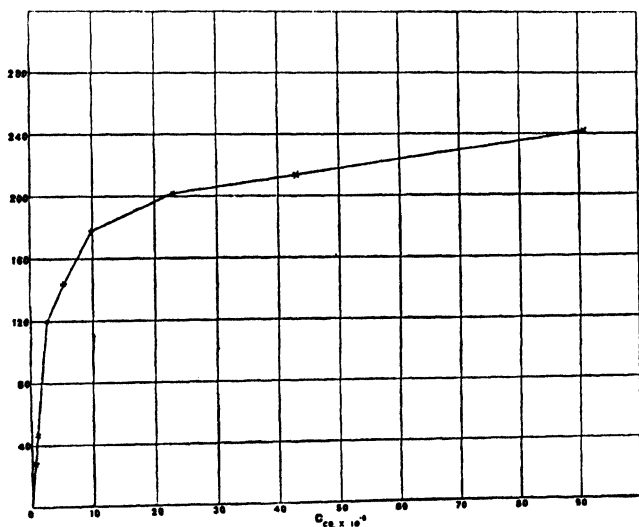


FIG. 23.—The rate of photosynthesis at different concentrations of carbon dioxide. The ordinate represents the rate of photosynthesis, abscissa the concentration of carbon dioxide. (Reconstructed from data given by Warburg. From Spoehr, *Photosynthesis*.)

directly proportional to the concentration. With progressive increase in the concentration the curve exhibited a continuously smaller increase in the photosynthetic rate until it appeared to be independent of the concentration of the carbon dioxide (Fig. 23). Warburg placed the following interpretation on his results: that the rate of assimilation is proportional (i.) to the concentration of the carbon dioxide, and (ii.) to the concentration of some second substance which reacts with the carbon dioxide.

These investigations of Harder and Warburg have been confirmed by James (1928), who found that moderately high con-

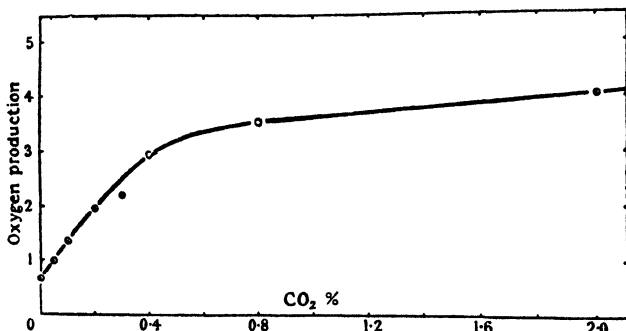


FIG. 24.—Curve of assimilation rate in 0—2.0 per cent. CO<sub>2</sub> at light intensity 20. (After James, *Proc. Roy. Soc. Lond.*)

centrations of bicarbonate or carbon dioxide solution depressed the assimilation rate of *Fontinalis antipyretica*. With a low light

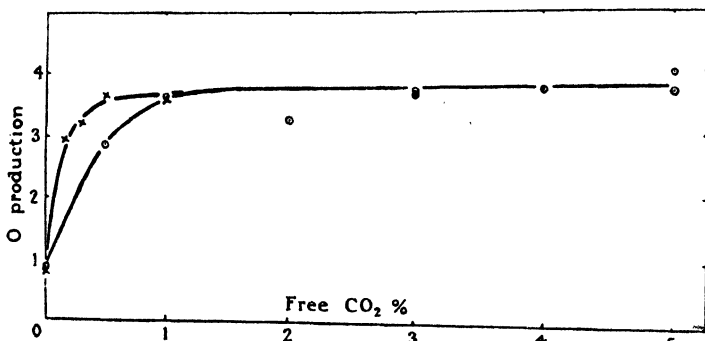


FIG. 25.—Rate of assimilation in solutions of 0—5.0 per cent. CO<sub>2</sub> moved through the apparatus at 400 and 600 c.c. per hour. Series O = 400 c.c. per hour. Series X = 600 c.c. per hour. (After James, *Proc. Roy. Soc. Lond.*)

intensity of 20 (arbitrary units) and at a temperature of 19° C. and low concentrations of carbon dioxide (0.05 to 2.00 per cent.), the curves obtained took the form of an oblique hyperbola (Fig. 24).

## CARBON ASSIMILATION

In higher concentrations of carbon dioxide (0.5 to 5.00 per cent.) and low light intensity (20), it was found that the rate of assimilation was independent of the concentration of carbon dioxide over the range 3 to 5 per cent. carbon dioxide (Fig. 25). Varying both light intensity and carbon dioxide concentration simultaneously, curves similar to Harder were obtained, *i.e.*, the factors were interdependent and not independent in their effect on the photosynthetic rate (Fig. 26). In these experiments carbon dioxide

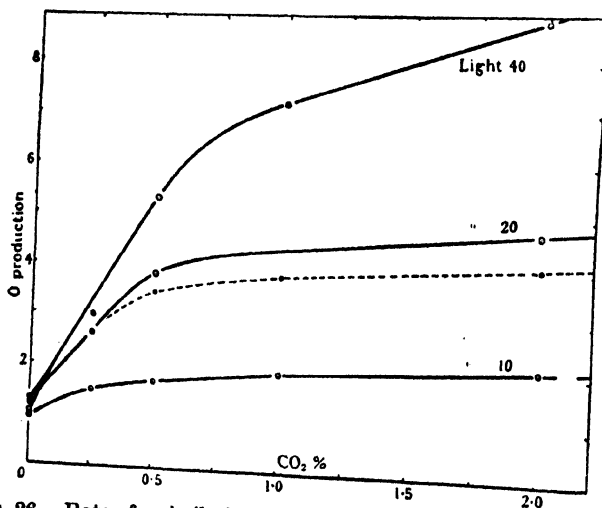


FIG. 26.—Rate of assimilation in CO<sub>2</sub> concentrations 0—2.0 per cent. and different light intensities (10 to 40). (After James, *Proc. Roy. Soc. Lond.*)

was supplied to the plant in solution, and the flow of liquid through the assimilation chamber was at a rate of 400 c.c. per hour. Experiments were also made with sodium bicarbonate as a source of carbon dioxide, and it was discovered that at the same rate of flow per hour (400 c.c.) the sodium bicarbonate gave rise to a higher rate of assimilation than a pure solution of carbon dioxide of equal partial pressure when no other factor was limiting. At a flow of 600 c.c. per hour with low light intensity and consequent slow assimilation, the two solutions gave the same assimilation.

It would seem that in bicarbonate solutions only the free carbon dioxide is available for photosynthesis. At a higher light intensity (80) the bicarbonate again gave rise to a faster rate of assimilation than a pure solution of carbon dioxide of equal partial pressure, though the increase of the rate of flow to 600 c.c. with bicarbonate solution at this light intensity did not increase the rate of assimilation.

James pointed out that the older investigators considered assimilation rate to be almost proportional to the concentration of carbon dioxide at low concentrations, but since a faster flow of liquid tends to make this factor disappear and return at higher light intensities, it is probable that the linearity of the curves obtained was due to the conditions of diffusion obtaining in their experiments rather than to internal stages in photosynthesis. The most acceptable idea is to consider the successive stages

in photosynthesis as being made up of a series of linked reactions, each member of the series being reversible.

The majority of the work on the validity or otherwise of the theory of limiting factors has been carried out on aquatics. Investigation of the problem in land plants is beset with a number of difficulties. It has been shown by Maskell (1928) that variations in the uptake of carbon dioxide by the leaf are due to variations in stomatal resistance and the remaining resistances of the leaf. The stomatal resistance is considered to depend on (1) the season of the year, the range of opening being different in

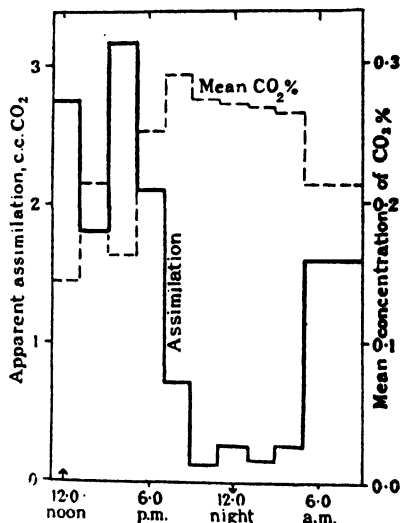


FIG. 27.—March of apparent assimilation compared with mean CO<sub>2</sub> concentration under continuous artificial illumination. (After Maskell, *Proc. Roy. Soc. Lond.*)

different months of the year under conditions of constant light intensity ; (2) time of day—there is a well-marked diurnal rhythm of stomatal opening at constant light intensity ; (3) time elapsing from the beginning of the experiment ; (4) intensity of illumination ; (5) previous history of the leaf ; (6) previous moisture conditions of the plant.

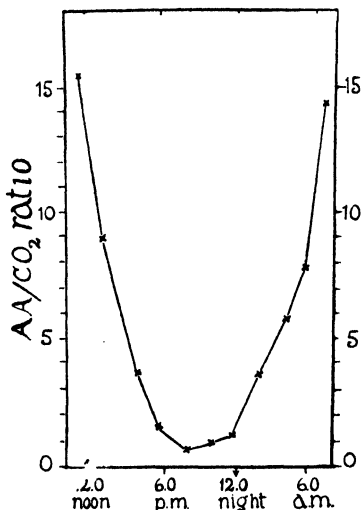


FIG. 28.—March of apparent assimilation in Cherry Laurel leaves under continuous artificial illumination. (After Maskell, Modified, *Proc. Roy. Soc. Lond.*)

Maskell, using *Prunus Lauro-cerasus* var. *rotundifolia* and the continuous current method of estimating assimilation, ascertained that there was a diurnal rhythm of photosynthesis with low concentrations of carbon dioxide and a constant intensity of light. The assimilation rate fell during the afternoon and evening, and rose again during the morning (Fig. 27). The apparent assimilation, rather than the real assimilation, was measured, as it was the uptake of carbon dioxide that was principally being investigated. A better picture of the march of assimilation is obtained by calculating for each reading the ratio

between the apparent assimilation and the mean carbon dioxide concentration, *i.e.*, the ratio :—

$$\frac{\text{A.A.}}{\text{CO}_2} = \frac{\text{Apparent Assimilation}}{\text{CO}_2 \text{ concentration}}$$

Simultaneous observations on the porometer and photosynthetic rate showed these results to be due to the march of stomatal aperture (Fig. 28).

In high concentrations of carbon dioxide, such that light instead of carbon dioxide was limiting the rate of assimilation, no diurnal rhythm was found. Moreover, besides this diurnal

rhythm, a seasonal rhythm was also discovered in the assimilation rate, and Maskell was able to show that the pitch or level of the diurnal photosynthetic curves was higher in July, August and September, than at any other times of the year.

Maskell accepted Warburg's picture (see below) of a photochemical phase in assimilation as a simple expression of a possible type of relationship between carbon dioxide concentration and light at the chloroplast surface. If this be so, then the rate of assimilation is proportional to :—

$$\frac{L \times C_s}{C_s + K_L}$$

where  $L$  is the light intensity,  $C_s$  is the concentration of carbon dioxide at the chloroplast surface, and  $K_L$  is the ratio of the velocity constant for the dissociation of some photochemical product (the rate of formation of which is proportional to  $L$ ) to the velocity constant for the combination of the photochemical product with carbon dioxide. Expressing the light intensity in terms of light limiting values of the real assimilation ( $y$ ), then :—

$$y = \frac{L \times C_s}{C_s + K_L}$$

If the resistance to the diffusion path of the respiratory carbon dioxide up to the chloroplast surface be small, or if the path be short compared with the path outside the leaf, then we have :—

$$\text{Assimilation (A)} = \frac{C - C_s}{D} = \frac{L \times C_s}{C_s + K_L} - R$$

where  $C$  represents the external concentration and  $D$  the total resistance of the leaf to the diffusion path of carbon dioxide from outside the leaf up to the chloroplast surface. Hence the real assimilation ( $y$ ), is :—

$$y = \frac{L \times C_s}{C_s + K_L} = \frac{C - C_s}{D} + R$$

from which :—

$$y = \frac{C}{D} - \frac{K_L}{D} \frac{y}{(l - y)} + R$$

and, for the curve relating  $y$  to increase in carbon dioxide concentration, we have :—

$$\frac{dy}{dC} = \frac{1}{D} \left( \frac{1}{1 + \frac{K_L}{D} \left( \frac{L}{(L-y)^2} \right)} \right)$$

when  $y = L$ , i.e., when the light limited value is recorded,  $\frac{dy}{dC} = 0$  or  $y = L$  forms the horizontal asymptote to the curve relating  $y$  and  $C$ . If  $y = \pm \infty$ ,  $\frac{dy}{dC} = \frac{1}{D}$ , this being the slope of the asymptote to the ascending limb of the curve. Similarly, for the curve relating assimilation and light intensity, we have :—

$$y = L \frac{\frac{C}{D} + R - y}{\frac{C}{D} + R - y + \frac{K_L}{D}}$$

then :—

$$\frac{dy}{dL} = \frac{\frac{C}{D} + R - y}{\frac{C}{D} + R - y + \frac{K_L}{D}}$$

when  $y = \frac{C}{D} + R$ , then  $\frac{dy}{dL} = 0$ . This gives the horizontal asymptote of the curve. When  $y = \pm \infty$ ,  $\frac{dy}{dL} = 1$ . This is the slope of the asymptote to the ascending limb of the curve.

Thus, for any given fraction of the maximal possible assimilation,  $\frac{C}{D} + R$ , at any given carbon dioxide concentration,  $\frac{dy}{dL}$  falls below unity according as  $D$ ,  $C$  or  $R$  are relatively small, or  $K_L$  is relatively great. That is to say, the corner of the curve relating light and assimilation will be sharp for relatively high concentrations and gradual for relatively low concentrations of  $D$ . If this equation forms any approximation to the real relationship between the factors controlling photosynthesis, the divergence found in the forms of curves relating assimilation to carbon dioxide concentration or light intensity represent variations due largely to

different relative values of the resistances in the diffusion and photochemical phases of the process.

The *Elodea* result of Blackman and A. M. Smith would correspond to a set of conditions of high resistance to diffusion or high deficiency of light intensity. On the other hand, Harder's results would correspond to low resistance to diffusion or low intensity of light at the chloroplast surface, or perhaps to both. Using such expressions, calculated and experimental results obtained by Maskell are given in Fig. 29. It will be seen that the general results obtained

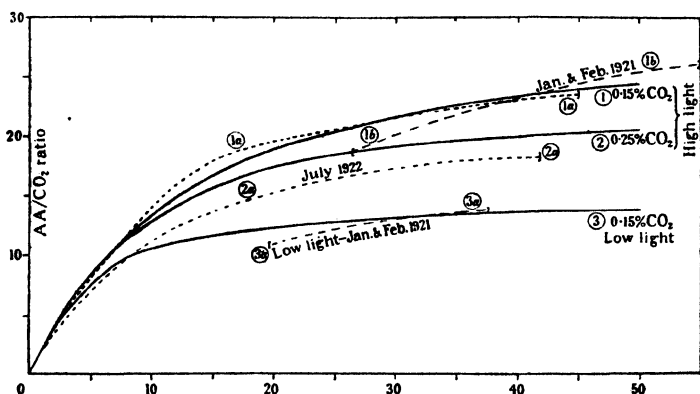


FIG. 29.—Theoretical relation between stomatal diffusive capacity and assimilation (mean CO<sub>2</sub> per cent. and light constant). Theoretical curves (1, 2, 3) are shown by the continuous lines. Experimental curves (1A, 1B, 2A, 3A) by broken lines. (After Maskell, *Proc. Roy. Soc. Lond.*)

from a consideration of the experimental data can be reproduced in the theoretical curves, and that the types of interaction found between apparent assimilation and stomatal opening, seasonal changes in photosynthetic activity and light intensity, follow naturally from the theoretical formula.

As Maskell very rightly pointed out, the original conception of the theory of limiting factors was only defined as a first approximation, and has been widely misconstrued as a rigid law "... and the term 'limiting factors' has been borrowed on all hands for the purpose of describing phenomena in *quasi*-quantitative

terms with the minimum of quantitative investigation . . . regarded and used as a clue to the interpretation of the phenomena, the general principle of limiting factors suggested by Blackman in 1905 cannot as yet be replaced."

### The First Sugar of Photosynthesis

The normal green plant in the presence of light and chlorophyll synthesises carbohydrates from carbon dioxide and water, and thereby converts radiant energy into chemical energy. The question arises, Which is the first sugar formed in the assimilating cells of the leaf? Sachs, in his classical experiments, showed that starch was a product of photosynthesis and demonstrated the fact that it was formed in the light and disappeared in the dark. Sachs described the starch as the "first visible product" of photosynthesis. Starch, however, is not always a product of photosynthesis. It is produced in storage organs, and in many plants, especially among monocotyledons; it is not normally found in the leaves, and only occurs to a small extent in the guard-cells of the stomata.

The starch molecule from the chemical standpoint is complex, and it is therefore unlikely that it is the first formed carbohydrate of assimilation, and it is probable on this ground alone that simpler sugars like the hexoses and disaccharides precede its formation.

It is not proposed to enter into a discussion here of the chemistry of the carbohydrates, as this has already been done in another member of this series (see Pryde, 1931). The monograph by Haworth (1929) should also be consulted in this connection as well as that by Onslow (1931).

The carbohydrates of the leaf have been submitted to numerous investigations, both qualitative and quantitative. The quantitative investigations of H. T. Brown and Morris in 1893 definitely established the fact that sugars were formed in the leaf as a result of assimilation. In the leaves of *Tropæolum majus* they found d-glucose, d-fructose, sucrose and maltose, but no pentoses. All subsequent investigations have failed to reveal the presence of

the *l*-isomerides of these sugars. Davis and Sawyer (1914) claimed to have discovered pentoses to be present in the leaves of the mangold. The presence of pentoses has been denied by other investigators. Their presence was inferred from the fact that furfural was obtained on distillation with 12 per cent. hydrochloric acid (Kröber's method). This method was employed in this work, but it has been shown to be inaccurate in the presence of other carbohydrates. Further, the presence of uronic acids, *i.e.*, carbohydrate derivatives with carboxyl and aldehydic group in the molecule, also give furfural on distillation with hydrochloric acid, and these substances have a wide distribution in the plant world (see Ling, Paton and Nanji, 1925). Davis, Daish and Sawyer (1916) were unable to confirm Brown and Morris's statement that maltose was present, and they considered that this result may well have been due to the hydrolysis of starch by diastase in the leaf cells as the material was not killed with sufficient rapidity.

Summarising these results up to the present point, it can be definitely stated that the products, either direct or indirect, of assimilation are: glucose, fructose, sucrose and starch. The question before us is this: Which sugar is produced first in the process? Such an investigation is difficult on experimental grounds. The amounts of these substances in the leaf are small, and unless the manipulations concerned with their isolation and estimation are carried out with extreme care they may suffer conversion into other products. Moreover, the interpretation of the experimental data is also beset with difficulties. The rates of change of these substances may be very different, and other complications are caused by translocation and respiration. On purely chemical grounds it would be expected that hexoses should be the first formed carbohydrates of photosynthesis. Yet the major number of workers who have investigated this particular problem of plant physiology have arrived at the conclusion that sucrose is the first sugar produced.

Two possibilities have to be considered in this connection: (a) the so-called up-grade sugars, synthesised from carbon dioxide and water; and (b) the down-grade sugars, produced by the

hydrolysis of reserve carbohydrates, such as starch, inulin and other polysaccharides.

Brown and Morris, from their investigations on *Tropæolum majus*, arrived at the result that since sucrose was always in excess of glucose and fructose in the leaf, sucrose must be the first sugar of carbon assimilation. The accumulation of fructose was explained as being due to the greater consumption of glucose in respiration. Excess of sucrose was considered to be converted into starch, while for purposes of translocation sucrose was inverted to glucose and fructose and removed as such. It was found after assimilation had been allowed to proceed all day, that the leaves contained no glucose and very little fructose, but considerable amounts of cane sugar were present. This was considered to lend further support to the view that sucrose must be the first sugar of photosynthesis. If translocation were prevented by cutting the petioles, then there was an increase in sucrose and starch, but not in glucose and fructose.

The technique employed, however, was faulty in many respects. Stiles and Jørgensen (1916) have pointed out in a critical discussion of this work that the values recorded by Brown and Morris for the glucose and fructose fractions of their preparations are extremely doubtful, while Davis, Daish and Sawyer (1916) showed that the large amounts of maltose found by Brown and Morris were due to the hydrolysis of starch by diastase. Gast (1917), on the other hand, also working with *Tropæolum majus*, in large measure confirmed the findings of Brown and Morris, and also found maltose to be present in the leaves. This latter confirmation was no doubt due to the fact that he employed the same method of killing the leaves, i.e., by heating them to 100° C. in an oven. Gast, however, displayed a considerable amount of caution in his final deductions, and did not commit himself to the view that sucrose is the first sugar of assimilation, but called it the "first analytically recognisable sugar." Parkin (1912), working on *Galanthus nivalis*, also held the view that sucrose is the first formed sugar in the leaf.

The work of Davis, Daish and Sawyer, briefly alluded to above, was an elaborate investigation on the mangold and potato to determine whether sucrose or hexose was the first sugar of photo-

synthesis, and up to the year 1916 was certainly the most important and authoritative pronouncement on this aspect of plant metabolism. It will therefore be necessary to describe this work in a certain amount of detail in order to clarify the ensuing discussion.

These workers estimated in the leaves, glucose, fructose, sucrose, maltose, starch, pentoses and pentosans. The leaf material was killed by throwing it into boiling 95 per cent. alcohol containing a little ammonia. After boiling for half an hour, the leaf material was extracted for from twelve to eighteen hours in a special Soxhlet extractor with the same alcohol. The alcoholic extract, after it had been reduced in volume by distillation under reduced pressure and cleared with lead acetate and made up to 500 c.c., was analysed for soluble sugars. Starch was estimated on the residue.

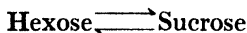
Collections of the leaves were made at two-hourly intervals over twenty-four-hour periods. Three such determinations were made—August, September and October. The mangold leaf was found to contain starch only in the early stages of growth. In each of these experimental runs the sugars of the leaf lamina and petiole were treated separately and the results expressed as a percentage of the total dry weight.

In general terms it was found that in the early summer the soluble sugars (hexoses and sucrose) increased in the light to a maximum about noon and then fell off fairly regularly till the following dawn, and the concentration of sucrose was always in excess of hexose, and the percentage of pentosans remained fairly constant. No maltose was found to be present. In the second experimental run (September) the hexoses were found to be in excess of sucrose and both curves showed maxima at 2 p.m., 6 p.m. and 2 a.m. The results obtained in October were much the same as those obtained in September.

The sampling error in this work was probably high. Davis, Daish and Sawyer attempted to overcome this difficulty by using large samples for each determination (2 kgm.). But even in these circumstances they give values for sucrose by the copper reduction and optical rotation methods that differed by 20 per cent., and estimations of hexose and sucrose in two samples gathered at the same time differed by 6 per cent. Like Brown and Morris as well

as Parkin, Davis, Daish and Sawyer were of the opinion that sucrose was the first sugar of photosynthesis and that inversion of sucrose to glucose and fructose took place prior to translocation, since hexoses were found to be in excess of sucrose in the petiole. Davis, Daish and Sawyer were also the first investigators to suggest a possible mechanism for sucrose formation from carbon dioxide and water. They stated : " It would seem, indeed, that plant leaves in general possess in the chloroplasts a mechanism for elaborating cane sugar directly from the carbon dioxide of the air." A further argument advanced by Davis, Daish and Sawyer for sucrose as the first sugar, based on the well-known enol conversion of glucose to fructose and mannose, and the absence of mannose from leaves, does not in fact apply. The enolic form of glucose can only exist in alkaline solution, whereas the reactions in the leaf are taking place in acid media.

Although under the analytical conditions employed by these workers there can be no doubt that sucrose was at certain times in the season in excess of hexose in the leaves, yet it would seem to be a misinterpretation of experimental data to conclude that sucrose is therefore the first sugar of photosynthesis. Chemically speaking, it is very unlikely that such a state of affairs should exist. As V. H. Blackman has pointed out, if formaldehyde be the initial product of carbon assimilation, it is more probable that the aldehyde should straightway be polymerised to hexose rather than to a disaccharide like sucrose. Blackman therefore suggested that a hexose is first formed and that a state of equilibrium exists between hexose and sucrose :—



when the concentration of the hexose reaches a certain value it is converted into sucrose. Hence the value of the hexose is practically constant in amount, and this is the condition which has been found to exist. If there be a fall in the value of the hexose, then the sucrose is hydrolysed back to hexose, so that the hexose still remains constant in amount.

Priestley (1924) took very much the same standpoint. He considered that since sugars are formed as intermediate steps in

the formation of more complex anhydrides, such as starch, it is necessary that these reactions should take place smoothly and rapidly: "It is to be expected then that the intermediate stages in the process, including the sugars first formed in photosynthesis, instead of accumulating in the light and therefore fluctuating in amount, should pass rapidly into other substances in the complex chain of metabolic changes so that little if any change in their concentration can be detected, and in any case no such phenomenon would occur as a local accumulation such as is characteristic of a storage product" (Priestley).

With regard to the function of sucrose in plants Priestley has put forward an unusual suggestion, namely, that sucrose is a by-product of ageing protoplasm in meristematic cells and that as these cells become vacuolated sucrose is released. It would thus appear from this view that sucrose at the growing meristems of the plant arises as a catabolic product. As Clements (1930) has pointed out, this view at once eliminates not only the places of storage, as, for example, in the sugar cane and sugar beet, but more important still also eliminates the leaves. It may well be asked how the sucrose is formed in these regions.

As Parkin (1925) pointed out, cane sugar is a product peculiar to the vegetable kingdom. It has a wide distribution and is found in every group of green plants. Kylin was unable to detect the presence of sucrose in the Phaeophyceæ, but stated that glucose was present. P. Haas and Hill (1929, 1931, 1932, 1933) have made a careful investigation of the carbohydrates present in certain members of the Phaeophyceæ. In *Pelvetia canaliculata* f. *libera*, *Fucus serratus* and *Ascophyllum nodosum* they found a small amount of free pentose to be present, although they themselves admit that the figures are scarcely significant. In *P. canaliculata* f. *libera* a small amount of a pentose complex, probably a disaccharide, was also found to be present, which only reduced Fehling's solution after hydrolysis. Ricard (1931) has also been unable to find either glucose or fructose in the free state in *Laminaria flexicaulis* and *L. saccharina*. Haas and Hill state that the sugar alcohol mannitol appears to be of universal occurrence in the Phaeophyceæ, and it may perhaps play a definite

metabolic rôle in these plants. In one member of the Rhodophyceæ (*Bostrychia*) they discovered the presence of the sugar alcohols, dolcitol and sorbitol. In the present state of our knowledge it is difficult to give any definite opinion as to whether mannitol in the Phæophyceæ is a primary photosynthetic product, and that the carbon metabolism of these plants is based on alcohol rather than sugar. If this be the case, the sugar found to be present is possibly an oxidation product of mannitol.

In the mammary gland, glucose previously stored as glycogen travels through the blood stream and is converted into lactose, and the following equilibrium exists in the system :—



Parkin has tentatively suggested that perhaps the equilibrium :—



exists in the plant. Chapman (1925) considered that the system is very much more likely to be :—



and that the reaction velocity is greater in the second reaction than the first. Again, the reverse series of changes should also hold :—



and in this case the reaction velocity of the glucose  $\rightleftharpoons$  maltose system would be greater than the maltose  $\rightleftharpoons$  starch system.

The evidence at present available, however, shows that sucrose and starch in certain circumstances are interrelated and maltose plays no part in the matter.

The problem of the first sugar has been investigated from a different aspect by Weevers (1924). Realising the difficulties of the earlier investigators who all used green leaves, he employed variegated leaves of a number of species instead, and estimated the sugar content of the green and non-green portions. In *Acer Negundo*, *Hedera Helix*, *Humulus Lupulus*, *Pelargonium zonale* and a number of other plants, he ascertained the presence of both

sucrose and hexose in the green portions of the leaves and only sucrose in the non-green parts. Two exceptions to this rule were discovered : *Cornus sanguinea* and *Æsculus Hippocastanum*. Here hexose was also found in the non-green portions, but only in very small amount. Since the variegated portions of these plants contain no chlorophyll and photosynthesis is impossible in these regions, it would seem a significant fact that no reducing sugars were found in these parts. The evidence from this fact alone, however, is by no means conclusive, but in a second experiment Weevers found that the leaves of the variegated form of *Pelargonium zonale* when placed in the dark for forty-eight hours were completely deprived of all sugars (a fact which a former colleague of mine, Mr. M. C. Pratt, has been able to confirm). At the end of this period the plants were allowed to assimilate, and it was discovered that the first sugar formed was a hexose and not sucrose. The following values were recorded :—

After $\frac{1}{2}$ hr. insolation (per 10 gm. dry wt.)				Traces of reducing sugars.
				No sucrose.
„ 1 „	„	0.3 per cent.	hexose	Traces of sucrose.
„ 3 hrs.	„	0.4	„ „	0.3 per cent. sucrose.
„ 5 „	„	0.6	„ „	0.3 „ „

If this experiment be correct, there can be little doubt that a hexose precedes the formation of sucrose in the green plant.

Tottingham and his co-workers (1926) showed that in the leaf-blade of the sugar mangold and sugar beet, sugars increase with the solar radiation within certain limits. A temperature, for example, of 30° C. acts as a limiting factor on the increased production of reducing sugars in the presence of high illumination. The sucrose content was found to vary very irregularly in the leaves and over a considerable proportion of the period investigated followed the course of the reducing sugars. Occasionally, however, it varied inversely as the hexose content. In view of these results, hexose and not sucrose was claimed to be the primary product of the assimilation of the leaf.

More recently Barton-Wright and Pratt (1930b) have investigated the question of the first sugar of photosynthesis in the

daffodil (*Narcissus Pseudo-Narcissus*). The *Narcissus* leaf, like the greater number of monocotyledonous leaves, only forms hexoses and sucrose and contains but a negligible amount of starch in the guard-cells of the stomata, so that the complications introduced into the problem by the presence of starch are obviated and the matter is considerably simplified in comparison with carbohydrate formation in dicotyledonous leaves.

It was discovered that the method of killing leaf material with boiling alcohol (*cf.* Davis, Daish and Sawyer) is not to be recommended, as considerable amounts of formaldehyde are produced with commercial alcohol and acetaldehyde when absolute alcohol diluted to 95 per cent. is used. This fact applies not only to the *Narcissus* leaf, but also to dicotyledonous leaves. The presence of either formaldehyde or acetaldehyde in the leaf-sugar preparations will vitiate hexose determinations, and it was shown by Barton-Wright and Pratt that not only is the copper reducing power of the solution affected, but the optical rotation of sugars is also altered by the presence of acetaldehyde. Thus, the figures given by Davis, Daish and Sawyer for hexoses must be looked upon with suspicion.

The method eventually employed was to kill the material by throwing it into boiling water containing a little ammonia to neutralise organic acids, after a preliminary freezing of the tissue at  $-16^{\circ}\text{C}$ . for six hours.

The sugars of the leaf were investigated at different times in the growing season (March 31st, May 8th and May 20th). Samples were gathered at hourly intervals and the results expressed as a percentage of the "residual dry weight," *i.e.*, dry weight less total sugars (see Chapter V). The first experimental run, while giving no definite evidence for hexose as the first sugar of assimilation, nevertheless confirmed the earlier observations of Parkin that sucrose is in excess of hexose before the flowering period. It also showed that it is improbable that sucrose is the first sugar of photosynthesis, as it was found that the maximum values for sucrose are only attained after a number of hours of assimilation, and, further, the rise in sucrose concentration is unaffected by the presence of rain.

In the second experimental run, which was of short duration (four hours), and was made after the flower buds had opened, it was found that hexose is now in excess of sucrose in the leaves, a result again in agreement with Parkin and other workers. The experimental run commenced at 9 a.m., after the plants had been in the dark for twenty-four hours. Over the first hour hexose

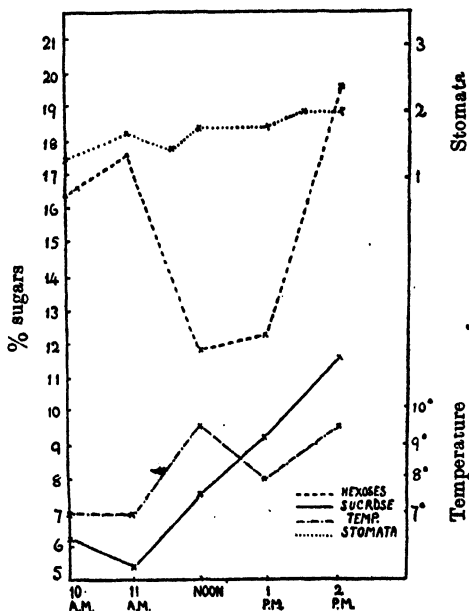


FIG. 30.—Curves showing fluctuations in hexose and sucrose in the leaf of *Narcissus*. Note the fall in hexose during rain, while sucrose remains unaffected. (After Barton-Wright and Pratt, *Biochem. J.*)

increased, while sucrose fell. But from 10 a.m. onwards to 1 p.m., there was a continuous rise in sucrose (Fig. 30). At 10 a.m. there was a particularly heavy shower of rain and decrease in light intensity, and during this time the hexose value dropped by nearly 6 per cent. Sucrose, on the other hand, was not affected and rose steadily during this rainy period. Although this experiment yielded very definite evidence for hexose, being the first

sugar of photosynthesis, the final experimental run which extended over a period of nineteen hours, gave still further support for the view that hexose and not sucrose is the first sugar of carbon assimilation.

Continuous records were made of temperature, humidity and stomatal aperture in this experimental run, and the results, graphically expressed, are shown in Fig. 31. The data were statistically treated and it was found that the correlation co-

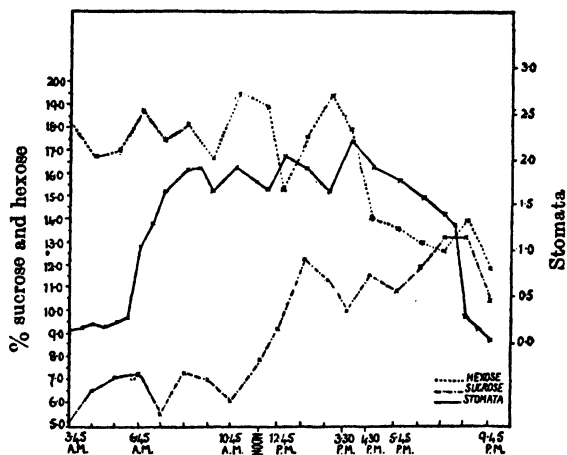


FIG. 31.—Curves showing fluctuations in hexose and sucrose in the *Narcissus* leaf. Note the gradual rise to a maximum in the sucrose curve. (After Barton-Wright and Pratt, *Biochem. J.*)

efficient for hexose and sucrose was significant ( $r = +0.7100$ ). The correlation coefficient for hexose and temperature ( $r = +0.4700$ ), though not quite significant, was higher than that for sucrose, and temperature ( $r = +0.3000$ ). It was also discovered that there was a negative drift with time in the hexose values (hexose/time,  $r = -0.8400$ ), whereas there was a positive drift with time for the sucrose percentages (sucrose/time,  $r = +0.8750$ ). Examination of Fig. 31 shows that there were marked fluctuations in hexose, whereas sucrose only began to rise continuously after 10.45 a.m., *i.e.*, increase in sucrose lagged behind increase in hexose. Since a

lag was discovered between increase in hexose and increase in sucrose, these authors shifted the values on between hexose and sucrose in order to determine whether this would bring about any increase in the correlation coefficient. The result of making a shift of four hours increased the correlation from  $r = +0.7100$  to  $r = +0.7700$ . Variations in hexose are therefore reflected in sucrose and the lag period is significant. Barton-Wright and Pratt held that this and other data presented by them support the view that hexose and not sucrose is the first sugar of carbon assimilation. There is in the first place a direct correlation between hexose and sucrose. There is also a greater correlation between hexose and temperature than between sucrose and temperature. It is true that the former correlation is barely statistically significant, but, on the other hand, plants such as the *Narcissus* and other bulbous monocotyledons are in active growth in the cold seasons of the year, and it is therefore possible that temperature does not play such a large part in their metabolic activities as in summer vegetation. There is a significant lag between increase in hexose and increase in sucrose, and even in the case in which sucrose was in excess of hexose in the leaf, *i.e.*, before flowering, the sucrose values only gradually drifted towards a maximum value.

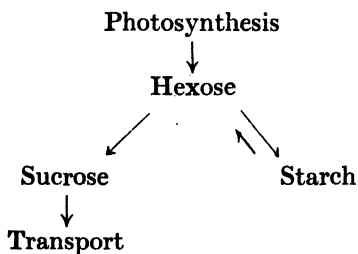
Examinations of the carbohydrate fractions of different dicotyledonous leaves at hourly intervals over twenty-four-hour periods have also been conducted by Clements (1930), working in America. The plants used were *Helianthus annuus*, *Soja max* and the potato (*Solanum tuberosum*). Clements also considered that his data supported the view that "simple sugars" (hexose and pentoses) precede the formation of sucrose in the leaf, and that the latter serves the function of a temporary storage product and is also the mobile form of sugar in the plant.

A comparison of the carbohydrate metabolism of normal healthy potato plants with that of potato plants affected with the virus disease known as leaf-roll has been carried out by Barton-Wright and M'Bain (1932). Here, again, it was considered that the data supported the view that hexose and not sucrose was the first formed sugar in the leaf.

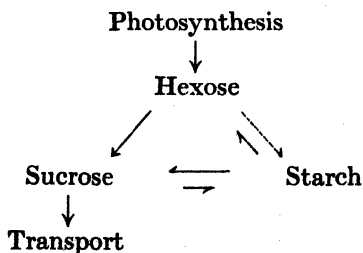
In the healthy potato it was found in the early part of the

growing season (May) that sucrose is in excess of hexose, whereas later in the season hexose is greater in amount than sucrose. The data principally relied upon in this connection by these authors is much the same as that given for the *Narcissus* leaf, namely, negative drift with time for hexose and positive drift with time for sucrose, and lag between increase in hexose and increase in sucrose. It was also found from the regression equations calculated for the results that increase in sucrose is dependent on decrease in hexose, and the reverse reaction is of no great extent, and, finally, the relation between temperature and hexose is statistically significant, whereas that between sucrose and temperature is not.

It was also found that under certain conditions sucrose could be formed from starch. For example, in high light intensity it was considered that the following reactions took place :—



whereas if the light intensity were poor the relationships between the carbohydrates were interpreted as :



Later in the growing season there is a variation in the carbohydrate metabolism. The sucrose values fall considerably and

there is an increase in the hexose percentages. Starch formation is the principal reaction taking place in the leaf, and the starch is formed from two different sources, hexose and sucrose, sucrose having relatively the greater influence.

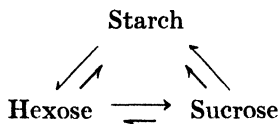
It is a well-known fact that leaves which normally form starch, when floated on solutions of different sugars in the dark, such as glucose, fructose, galactose and sucrose, can be induced to form starch. With very few exceptions, the results show that sucrose is the best starch former, galactose having the least effect.

There are at present but few investigations available with regard to the formation of sucrose from starch and *vice versa*. Ahrns (1924) has put forward the claim that both hexose and sucrose increase in concentration when different leaves are desiccated. This is certainly a curious result, as the presence of water in *in vitro* experiments accelerates enzymic hydrolysis. Schroeder and Horn (1922) have obtained similar results, although they state that increase or decrease of sucrose is independent of the amount of hexose present.

The formation of sucrose in potato tubers has been investigated by de Wolff (1926a, 1926b), who found that if slices of tuber were artificially dried the sugar content increases and this increase is definitely due to sucrose, the hexose remaining approximately constant. He also adduced evidence to show that on the addition of water to the dried material there was a corresponding decrease of sucrose. From his investigations, de Wolff concluded that the conversion of starch to sucrose is a reversible reaction, but that it takes place through several stages. Barton-Wright and M'Bain ascertained that in healthy potato leaves the reaction between sucrose and starch is mainly in the direction starch  $\rightarrow$  sucrose. Since sucrose is apparently the sugar of transport (see Chapter V), there will be a continuous export of sucrose from the lamina to the tubers, and it is possibly on this account that the sucrose concentrations never reach the requisite level for the reverse reaction sucrose  $\rightarrow$  starch to take place in any marked degree in the leaf, although in the later part of the season this is the case.

In the laminae of leaf-roll plants it was found that the relationship between sucrose and starch is particularly close. Leaf-roll

is a disease which causes marked accumulation of starch in the laminae of potato plants, and the suggestion was put forward by these authors that in the early stages of the disease photosynthesis is only of small amount and that the following reactions are taking place in the leaf-blade :



*i.e.*, a series of interconversions in a practically closed system, for in leaf-roll plants the channels of transport are disrupted.

Barton-Wright and M'Bain put forward the following suggestions for the carbohydrate reactions in the healthy potato plant : "Photosynthesis leads to the formation of hexoses, probably the active  $\gamma$ -forms, and that the subsequent reactions are dependent on light intensity. Under high light intensity the reaction is from hexose  $\rightarrow$  sucrose, and this reaction is to no marked degree reversible. There is also condensation of hexose to starch, probably as Onslow suggests, by condensation of  $\gamma$ -glucose with normal glucose, and this reaction is reversible, but mainly in the direction of hexose  $\rightarrow$  starch, during the period of assimilation. Further, we suggest that this reaction comes into play when the concentration of sucrose reaches too high a concentration for rapid removal by translocation, and the formation of insoluble starch prevents harmful osmotic effects from too high concentrations of soluble sugars. Under low light intensity there is still condensation of hexose to sucrose and at the same time hydrolysis of starch to sucrose. The sucrose in these circumstances has a dual source of origin, again to supply the necessary concentrations for translocation to the tubers. When the light intensity is low, the reaction hexose  $\rightarrow$  starch is not proceeding to nearly the same degree as when light intensity is high. The function of starch in the lamina, then, is that of a temporary storage product, and its hydrolysis leads to the formation of sucrose, which is the translocatory sugar in normal green plants."

The formation of carbohydrates in potatoes at senescence has

also been investigated by Barton-Wright and M'Bain (1933), who have found that at this stage photosynthesis is very small in amount, and increase in hexose is due to hydrolysis of starch, and the hexose in turn is condensed to sucrose.

These authors make the suggestion that since at senescence photosynthesis has practically come to a standstill, and hexose formation is now dependent upon starch hydrolysis, that the fall in sucrose which has been observed to occur by numerous investigators late in the growing season can be explained. Since the evidence at present available shows that sucrose is the principal sugar of transport, and as sucrose is formed from hexose and starch, with decrease in photosynthesis, there will be a corresponding decrease in the formation of hexose. At the same time, however, translocation is continuing and sucrose is being removed from the leaf. In these circumstances, if the rate of transport of sucrose out of the lamina be greater than its rate of formation from hexose, the sucrose values will fall, and it may be on this account that the sucrose percentages recorded for different plants have shown a fall late in the growing season.

If a hexose and not sucrose be the first formed product of photosynthesis, a further difficulty has to be contended with, for not one, but two hexoses, glucose and fructose, must be produced to give cane sugar by condensation. In this connection Nef (1913) showed that aqueous solutions of *d*-glucose, *d*-fructose, and *d*-mannose, in the presence of a 0.05 equivalent of calcium hydroxide at the ordinary temperature, gave mixtures composed of *d*-mannose, *d*-glucose, *d*-fructose, *d*-pseudofructose, as well as  $\alpha$ -,  $\beta$ -, and *d*-glutose. Similar results have been obtained by Spoehr and Wilbur, who have been able to bring about interconversion of sugars in the presence of solutions of disodium hydrogen phosphate, as well as in neutral mixtures of this salt and sodium dihydrogen phosphate. Again, in the animal economy, fructose is converted into glucose. On these grounds, if glucose be first produced in the leaf, the mesophyll might be possessed of a special and at present unknown mechanism to convert a part of the glucose into fructose and other hexoses. Nevertheless, mannose has never been isolated as such from the green leaf; its recognition should be

simple, as it is the only known hexose which forms a sparingly soluble hydrazone. In this connection, Clements (1932) has investigated forty-two different species of plants from various groups, *e.g.*, Angiosperms, Gymnosperms and Pteridophytes, in an effort to determine whether mannose is present in the leaves. In no case could a trace of mannose be discovered. It would thus seem that this hexose plays no part in the photosynthetic scheme. Clements suggests that it is probable that fructose is formed independently of glucose in leaves from carbon dioxide and water, for unless the stereo-chemical relationships known to exist among glucose, fructose, and mannose, are different in the cell sap of the plant from what they are in pure water, it is to be expected that were fructose formed from glucose, mannose would also be present, and this is not the case.

### The Chemical Mechanism of Photosynthesis

The chemical mechanism of photosynthesis has led to almost endless speculation and no very convincing proof. It is obvious that the process takes place in stages, and that the first stage is one of reduction. The main question that has to be decided is whether this reduction is brought about by photochemical means, or, if it be due, as Warburg held, to the production of some substance in the cell, which, under the influence of light, is capable of reducing carbon dioxide.

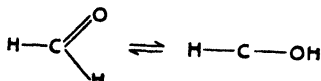
It must be emphasised that carbon assimilation is an endothermic reaction, that is to say, a reaction in which heat is absorbed. The sugars produced, whether hexoses or disaccharides, are compounds of higher potential energy than the initial substances, carbon dioxide and water. If photosynthesis were a purely photochemical reaction, then the Van't Hoff coefficient,  $Q_{10}$ , would be less than 2.0. Matthaei found the value to be approximately 2.0. It follows, therefore, that some other purely chemical reaction or possibly even some enzymic reaction is involved as well.

The main theory of the chemical reactions involved in photosynthesis and the one that seems to have fascinated the minds of nearly all subsequent investigators, is that put forward as far

back as 1870 by the German chemist, Baeyer. Struck by Butlerow's experiments on the action of calcium hydroxide on solutions of formaldehyde in which a complex mixture of sugars were produced, Baeyer suggested that the first product formed in assimilation from carbon dioxide and water was formaldehyde, and that this was subsequently polymerised to sugars. The theory has been tested by innumerable investigators, and it must be confessed that the majority have pronounced in its favour on no very adequate grounds.

The most recent support for Baeyer's hypothesis emanates from the laboratory of Professor E. C. C. Baly, who with his co-workers has published a number of papers on the subject which will be considered here.

In the first communication, Baly, Heilbron and Barker (1921), pointed out the well-known fact that carbon dioxide absorbs light of wave-length  $200\ \mu\mu$ , while formaldehyde absorbs light of a wave-length  $290\ \mu\mu$ . Hence the suggestion was put forward that if photosynthesis were to take place, it would occur in two stages : (1) the formation of the aldehyde from carbon dioxide and water, which would require light of wave-length  $200\ \mu\mu$ ; and (2) the subsequent polymerisation of this aldehyde, which would require light of wave-length  $290\ \mu\mu$ . These workers considered that ordinary formaldehyde, which is a highly toxic substance to the plant, is not produced, but the tautomeric form of the aldehyde is formed in the leaf :



and it is this "active" aldehyde that is polymerised to reducing sugars.

This early work of Baly and his co-workers led to a storm of criticism. Spoehr was quite unable to find any evidence of the production of formaldehyde from carbon dioxide and water on illumination with a quartz mercury vapour lamp as Baly laid claim to have done. Baly replied that Spoehr must have used the

wrong shape of lamp! Irvine and Francis (1924) examined the mixture obtained by Baly, on exposure of solutions of formaldehyde to ultra-violet light, and stated that the syrup appeared to contain 25 per cent. of sugar (calculated as glucose), but the amount was reduced when the solution was heated with acid. Methylation appeared to show that 9.3 per cent. of sugar was present in the mixture, while the bulk of the material was definitely a non-sugar in nature, but contained hydroxyl groups. Porter and Ramsperger (1925) reported that illuminated solutions of carbon dioxide form formaldehyde when they come into contact with sealing wax, cork or rubber, whereas, if the entire apparatus be constructed of quartz, no aldehyde is produced.

The very active criticism aroused by his work has made Baly shift his original position very considerably, and he now holds that it is unnecessary to postulate two separate phases for photosynthesis, and that the activated aldehyde produced from carbon dioxide and water can polymerise itself to reducing sugars without loss of energy and subsequent reactivation.

According to Baly, Davis, Johnston and Shanassy (1927), when an aqueous solution of carbon dioxide is exposed to ultra-violet light, a so-called photostationary state is produced. Ordinary formaldehyde is not a component of this system of equilibrium, but there is present a component which is probably a complex aldehyde. They found that ferrous bicarbonate in aqueous solution in the presence of ultra-violet light and in the absence of oxygen was converted into ferric hydroxide with the simultaneous formation of organic compounds with reducing properties. This reaction appeared to take place on the surface of the quartz tubes employed in the experiment. They further found that when insoluble barium sulphate and metallic aluminium were suspended in water through which a stream of carbon dioxide was passing, and the whole was exposed to ultra-violet light, complex organic compounds of the nature of carbohydrates were produced. In still further experiments on this subject, Baly, Stevens and Hood (1927) discovered that carbon dioxide adsorbed on the surface of either nickel or cobalt carbonate suspended in water, photosynthesised organic compounds in the presence of ordinary light.

The amount of photosynthesis in the presence of coloured catalysts was greater than in the presence of white powders like barium sulphate. On this ground, therefore, Baly and Davis (1927) claimed that there is a marked similarity between photosynthesis *in vivo* and *in vitro*, and that the following features appear to be common to both :—

1. Ordinary formaldehyde does not take part in either.
2. The laboratory process is realised by the action of carbonic acid on a surface. In the plant it takes place on the surface of the chloroplasts.
3. A visibly coloured surface and visible light function in both.

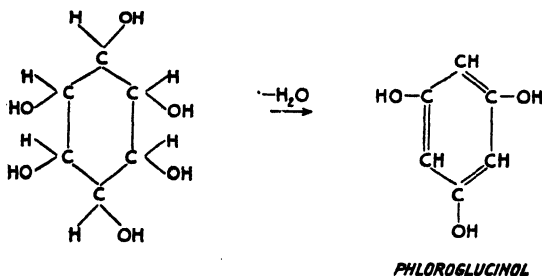
Baly's latest view, therefore, appears to be that the theory as originally advanced was wrong. The mistake lay in supposing that light of two different wave-lengths was necessary in the process. This is now thought to be quite unnecessary, and that there is only one stage. The carbonic acid absorbs light and becomes activated, at the same time losing oxygen to give active aldehyde ( $\text{H}-\text{C}-\text{OH}$ ). A photostationary state occurs when pure carbon dioxide is exposed to ultra-violet light :—



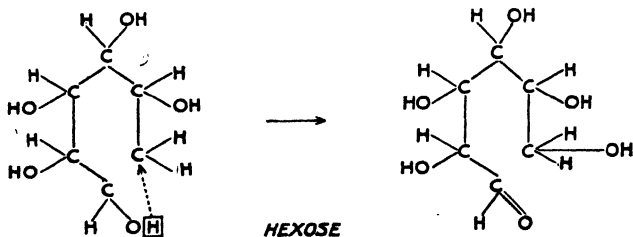
This photostationary state is considered to explain the results obtained by Porter and Ramsperger with apparatus entirely constructed of quartz. Any kind of reducing substance present will take up the oxygen, for example, iron or aluminium, and suffer oxidation. Again, with coloured compounds such as the salts of nickel and cobalt, this reaction will take place in ordinary light on the surface of the catalysts. In the plant, the carbon dioxide is supposed to combine with the surface of the chloroplasts, and the action of the chlorophyll is considered to be analogous with that of the coloured catalysts nickel and cobalt carbonate. It is the agent which causes the transformation of the active region of the light from the ultra-violet to the visible.

With regard to the polymerisation of the active aldehyde, ( $\text{H}-\text{C}-\text{OH}$ ), the carbon will tend to realise two desires : (1) to become tetravalent, and (2) to take the most stable configuration.

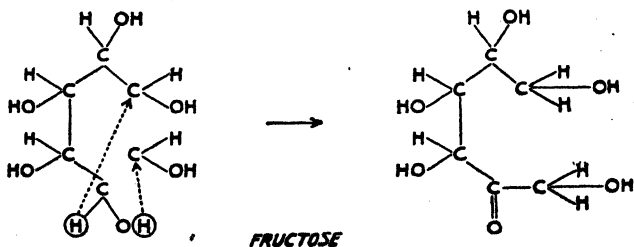
If polymerisation give a six-ringed compound, both desires will be simultaneously realised and satisfied :—



A further possibility is the wandering of one of the hydrogen atoms of a hydroxyl group to an adjacent carbon atom with the breaking of the bond between the two carbon atoms :—



This is the constitution of a hexose. If we imagine two hydrogen atoms to wander, then the result would be fructose :—



This, however, is a suggestion with no experimental proof.

Baly and Hood (1929) claimed that temperature has the same effect on their *in vitro* experiments as in the living cell. They

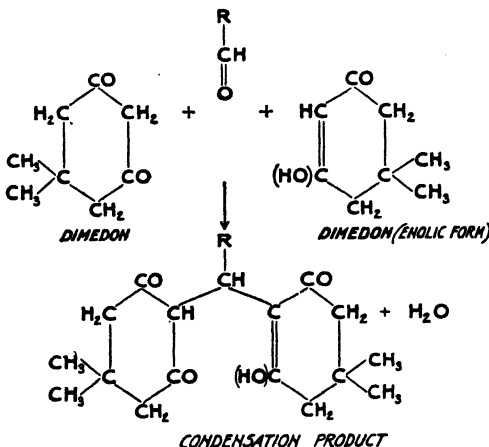
determined the yield of carbohydrate at different temperatures, using nickel carbonate as a catalyst for the reaction. A linear relation was found to hold between 5° C. and 31° C., and there was a rapid decrease in yield at higher temperatures than this. Although the relationship is not quite linear in the case of the leaf, they attributed this to the following causes : the simultaneous production of protein, and the orientation of the chloroplast to light may be different at higher temperatures.

It must be remembered that the whole of Baly's work has been conducted by means of *in vitro* experiments, and the plant has never been considered. It cannot be too strongly emphasised that the plant is not a test-tube. Because certain reactions take place in a certain way in the laboratory, it does not follow in the least that they can take place in that particular manner in the living cell. The chemical reactions of the mesophyll tissues of the leaf are complex in the extreme, and it is somewhat absurd to compare the rough-and-tumble heroics of Baly's test-tube experiments with the marked smoothness of the processes of the photosynthetic mechanism of the plant. Moreover, the numerous modifications that he has been forced from time to time to introduce into his original conception of the chemical mechanism of carbon assimilation, and the manner in which he has been dislodged from one untenable position only to take up another, allows one to place but little if any faith in the investigations of this worker.

Still less faith can now be placed in this work following on a recent publication by Bell (1931). Bell has reinvestigated Baly's work, especially that in which the latter claimed (1930, 1931) that satisfactory yields of carbohydrates could be obtained by using catalysts consisting of ferric hydroxide supported on kieselguhr which had previously been coated with alumina. Increased activity was claimed for the catalyst by the addition of small quantities of thorium dioxide. Bell entirely failed to substantiate this statement, nor was he able to confirm the work of Baly and Hood using nickel carbonate as a catalyst. Bell's experimental conditions were controlled with extreme care and specially pure carbon dioxide was used, but no trace of formalde-

hyde could be discovered, nor was there any conversion of carbon dioxide to carbohydrate compounds.

Klein and O. Werner (1926) have attempted to use the reagent dimedon (dimethyl-cyclo-hexanedione) to demonstrate that formaldehyde is a stage in photosynthesis. Dimedon reacts with aldehydes to give a condensation product :—



In the case of formaldehyde, formaldomedon is produced, and the crystals of this substance differ in shape and melting point from those obtained from acetaldehyde and dimedon, so that the two can be readily distinguished. In this investigation, aquatics such as *Elodea canadensis*, *Myriophyllum* and others, as well as mesophytes and succulents, were used. The greater part of the investigation was concerned with the assimilation of *E. canadensis*. Cut shoots of the plant were allowed to assimilate in nutrient solutions containing dimedon and calcium bicarbonate (to give a supply of carbon dioxide) for from six to eight hours in a light intensity of about 40,000 to 100,000 lux. Formaldomedon was subsequently found to be present in the *external* solution and but little in the plant. It was claimed that formaldomedon was only produced in the light and in the presence of carbon dioxide, whereas, in the dark, acetaldomedon was formed. Narcotics, such as phenylurethane and potassium cyanide, prevented the produc-

tion of the formaldehydon and only a small quantity of acetaldehydon was found. The presence of formaldehydon in the external solution and not in the assimilating tissues of the plant appeared to be a curious point, and on this account, this work was repeated by Barton-Wright and Pratt (1930a). The plant employed was again *Elodea canadensis*. It was found by these authors that bicarbonate solutions alone when exposed to light will give rise to formaldehyde. For example, it was shown that if a nutrient solution containing sodium bicarbonate and dimedon were exposed to light, as much formaldehydon was produced as when the living plant was present.

The further claim made by Klein and Werner that they were able to detect formaldehyde in the leaves of succulents and mesophytes either by direct injection of the dimedon or by conveying it to the assimilating organs *viâ* the transpiration current, is open to the objection that dimedon exerts a narcotic effect on the plant. Closure of the stomata, for example, takes place, so that the formaldehyde produced under these conditions may be a decomposition product.

Barton-Wright and Pratt sum the matter up thus : " Although the formaldehyde hypothesis has the merit of simplicity, which has probably been the prime cause of its wide popularity, no work has as yet convincingly shown that formaldehyde is produced normally in the green leaf or that it plays any part in the photosynthetic process of the living plant."

This statement is probably too sweeping. Since formaldehyde is toxic to the plant, it is hardly to be expected that it should accumulate to any marked extent in the free state under normal conditions. It may be assumed that, as soon as the reduction of carbonic acid takes place, the aldehyde formed is at once polymerised to sugar.

**Warburg's Theory.** Warburg (1919, 1920, 1921, 1922 ; Warburg and Uyésugi, 1924) has brought forward a theory of the mechanism of photosynthesis which is an improvement on any of those mentioned above, inasmuch that it takes into account the fact that the reaction is taking place in a living organism. He considered that there are several steps in the process, the first of

which is the so-called "photochemical primary reaction." In this reaction light is considered to act directly upon chlorophyll with the formation of a "photochemical primary product," the rate of formation of which is proportional to the amount of radiant energy absorbed per unit time. A second reaction now takes place between the photochemical primary product and an "acceptor." This acceptor is not carbonic acid itself, but is formed from the acid by a series of chemical reactions in the cell which are independent of the presence of light.

Warburg drew these conclusions from the fact that in high concentrations of carbon dioxide and high light intensity, the intensity of the assimilation rate does not increase with increase of these factors. Further, in such circumstances, with a rise of temperature of  $10^{\circ}\text{C}$ . the rate of photosynthesis is doubled, *i.e.*, the Van't Hoff law is followed. Warburg called this the "Blackman reaction." On the other hand, at low light intensities, the Van't Hoff coefficient,  $Q_{10}$ , is near unity; a result characteristic of photochemical reactions, and shows that light is playing some part in photosynthesis. Thus, with high light intensity,  $Q_{10} = 2$ , a value characteristic of ordinary chemical reactions, Warburg held that under these conditions a "dark reaction" is controlling the rate of photosynthesis.

Tsi-tung Li (1929) supported Warburg's view. He found an initial inhibition effect on the assimilation rate of a number of water plants when they were changed from light of high available energy for photosynthesis to one of low available energy. Similarly, there was an initial acceleration effect when the plant was moved from light of low available energy to one of high available energy. Li considered that the splitting off of oxygen does not take place in the primary photochemical reaction, but in a separate later reaction, which is possibly of enzymic nature. The actual degree of correlation between these two reactions varies with individual plants. For example, in a plant with a slow enzymic reaction, the initial inhibition and acceleration effects may be obscured. It is in many ways unfortunate that this author did not employ Wilmott's bubble-counting technique for his work, rather than the haphazard older method of counting the bubbles

that arise from a cut stem. The errors in the older method are so numerous that they might conceivably invalidate his conclusions.

Warburg also studied the effect of narcotics on the photosynthetic rate, and discovered that potassium cyanide inhibited the use of the carbon dioxide of the air for photosynthesis. When, however, the light intensity was reduced so that the respiratory rate exceeded that of assimilation, the rate of the latter was unaffected by concentrations of KCN of 0.02 M and under. In these circumstances the carbon dioxide of the air was not used for photosynthesis, but no carbon dioxide of respiration was released in the light, and, according to Warburg, either this respiratory carbon dioxide or perhaps some intermediate product of respiration is used in assimilation. Again, Warburg found that should a certain concentration of KCN inhibit the rate of photosynthesis by 50 per cent. at a high light intensity, a similar concentration will have no effect at low light intensity. This is taken as showing that the secondary reaction between the photochemical primary product and acceptor is unaffected by KCN, but that the Blackman or dark reaction is the one affected by the narcotic.

These results and views of Warburg have been obtained by the study of the living plant, and are on this account more to be relied upon than *in vitro* experiments. It is probable that the elucidation of the chemical mechanism of photosynthesis, which up to now has been such an elusive problem, will be eventually solved along lines of work similar to the investigations of this worker.

### The Chemistry of Chlorophyll

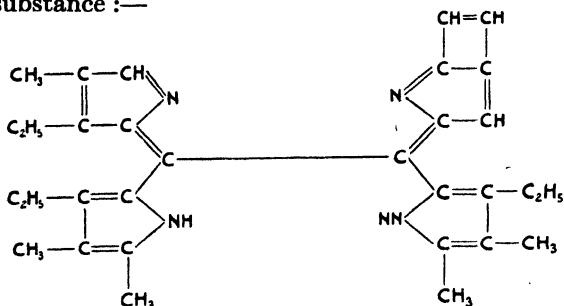
The work of Willstätter and Stoll, carried out over a number of years, showed that the pigments of the chloroplast are composed of four substances: chlorophyll *a*, chlorophyll *b*, carotin and xanthophyll. It is probable that xanthophyll is not a single substance but a mixture of closely related isomerides, and several investigators have claimed to have separated it into different fractions.

By the action of acids and alkalis, Willstätter and Stoll were able to show that chlorophyll *a*,  $C_{55}H_{72}O_5N_4Mg$  and chlorophyll *b*,  $C_{55}H_{70}O_6N_4Mg$ , were possessed of the same ultimate constitution, and that both gave the same magnesium-free body, *ætioporphyrin*,

$C_{31}H_{36}N_4$ . The steps whereby this substance was obtained are given in all the larger text-books of plant biochemistry (see P. Haas and Hill, "The Chemistry of Plant Products," Vol. I., 1928), and will not be considered here.

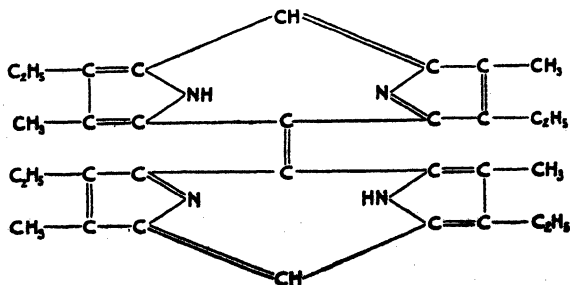
The ultimate composition of ætioporphyrin, however, is of great interest and importance, for it is probable that it is the groups within its molecule which are responsible for the characteristic properties of chlorophyll. It is also of interest on account of its possible relationship to the pigments of the blood.

The structure of ætioporphyrin was not established by Willstätter and Stoll, although they were able to show that it contained pyrrole rings, and they suggested the following constitution for this substance :—



*Ætioporphyrin (Willstätter and Stoll)*

H. Fischer and Klarer (1926) have now claimed to have established the constitution of ætioporphyrin by direct synthesis, and assign to it the following structure :—



*ÆTIOPORPHYRIN (FISCHER & KLARER)*

Conant and his co-workers (1929, 1930, 1931) have carried through a long series of investigations on the constitution of chlorophyll and have assigned structural formulæ to the various degradation products. The original papers should be consulted for an account of this interesting work as the results are too complex to receive adequate mention in a text of this nature.

### **Photosynthesis and Chlorophyll-content**

Willstätter and Stoll (1918) came to the conclusion that there was no relationship between photosynthesis and the chlorophyll content of leaves. There are a number of inherent difficulties in the use of leaves for an investigation of this nature. The temperature of the cells is never accurately known, and the palisade cells of dark-green leaves tend to screen the internal and lower cells, so that all are not equally illuminated.

This problem has been reinvestigated by Emerson (1929), using the unicellular alga *Chlorella vulgaris* to eliminate the difficulty of equal illumination. By varying the number of cells in a suspension, equality of illumination is readily controlled and the temperature of the cells is not significantly different from that of the surrounding medium which is easily kept constant.

The experiments were carried out at a constant temperature of 19°–21° C., and the illumination used was from a 40-watt lamp. The concentration of carbon dioxide was high, 5 per cent., so that the rate of photosynthesis was independent of small changes in carbon dioxide concentration. The assimilation was measured manometrically by Warburg's method of estimating the amount of oxygen evolved. Cultures were grown with varying amounts of ferrous sulphate, so that they developed a range of chlorophyll concentration. It was found that the respiration did not vary with the chlorophyll content and a uniform correction for respiration was made. It was found also that at high light intensities the rate of photosynthesis was a smooth function of chlorophyll concentration and the relation between the two was nearly linear. Finally, it was discovered that photosynthesis reached its

maximum rate at about the same light intensity, no matter what the chlorophyll content.

### The Chloroplasts

Photosynthesis, so far as we know, does not take place indiscriminately in the assimilating cells, but centres round certain bodies lying in the cytoplasm—the chloroplasts. It is on these plastid bodies that the chlorophyll pigments are fixed. The number of chloroplasts in cells varies very considerably and they also exhibit a great diversity in size and shape. Little is known as to how the chlorophyll is contained on the plastids. Treatment of the chloroplasts with alcohol removes the complex of pigments and leaves a colourless body—the stroma. The nature of the latter is also a matter of controversy; it is usually held to be of protein nature, and by some merely as a denser portion of the cytoplasm.

**Origin of the Chloroplasts.** It is usually asserted that chloroplasts arise from pre-existing chloroplasts. This is certainly true for the lower plants, *e.g.*, algæ, mosses and liverworts, in which their fate can be traced through the complete life-cycle. In the zygote of *Spirogyra*, however, they seem to lose their identity and make their reappearance at germination. In the higher plants there is a good deal of uncertainty as to their origin.

Chondriosomes or mitochondria, the small granules, threads or globules which are practically always present in the cytoplasm, have been considered by a number of workers to be the originators of plastids in the higher plants. Unfortunately our present knowledge of chondriosomes is very meagre and the literature highly conflicting.

In 1910, Lewitsky working on *Pisum* and *Asparagus*, concluded that the chondriosomes become leucoplasts in the root, and chloroplasts in the stem and leaves. It is difficult to assess the value of this work, but further evidence was brought forward on this point by Cavers (1914), Emsberger (1920), Friedrichs (1922), and Alvarado (1928). The most extensive work on this subject has been carried out by Guilliermond (1911-24). According to

this investigator, the chondriosomes only arise from pre-existing chondriosomes by division, a view at variance with other workers who consider that they may arise *de novo*. During the development of the plant they perform a variety of functions. Some of them form leucoplasts and chloroplasts, and also elaborate oils and other metabolic products. Mottier (1918, 1921) claimed to have found that in many liverworts and some higher plants, leucoplasts and chloroplasts are derived from rod-shaped chondriosomes, while there is also a second set of bodies which take part

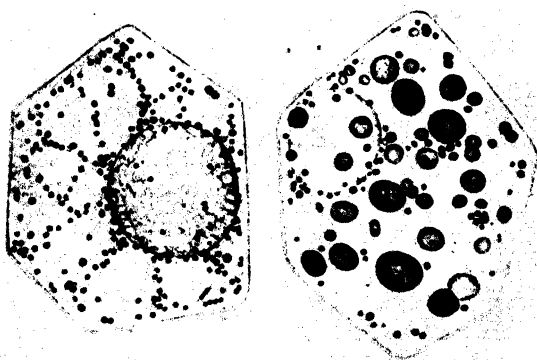


FIG. 32.—Left-hand figure, all from promeristematic region of a germinating seed of maize with small bodies showing as minute granules in the cytoplasm. Right-hand figure, later stage, showing the final stages in the maturing of the chloroplasts. (After Randolph, *Bot. Gaz.*)

in metabolism. Emsberger (1920, 1924) claimed to have found two similar classes in ferns, one of which forms plastids, and the other does not. Kirby (1928) considered that the chloroplasts in the spores of *Osmunda* are formed from chondriosomes or mitochondria.

Randolph (1922) has described the development of plastids from minute primordia in the cytoplasm, and uses the word "proplastids" for these bodies. He found that in the meristematic tissues of young stems and embryonic leaves of *Zea Mays*, all the cells contain these proplastids which appear as refractive

globules or granules of varying size, the smaller ones showing Brownian movement occasionally being carried about by the streaming of the cytoplasm. Concurrently with the differentiation of the cells, these proplastids gradually enlarge, develop chlorophyll, and are converted into chloroplasts (Fig. 32). Starch makes its appearance within them at a very early stage. Randolph was unable to discover the origin of these bodies, but he concluded that the smallest of these particles were submicroscopic in size.

Zirkle (1926) has studied the chloroplast in the living state, using *Selaginella*, *Elodea*, and a number of other plants. The material was frozen at  $-4^{\circ}\text{C}$ ., since it was found that freezing does not affect the chloroplasts. Below this temperature the chloroplasts appeared to liquefy and became spindle-shaped and fused into a green meshwork. After freezing, the chloroplasts were examined in monochromatic light of a known wave-length, as every pigment vanishes when examined in light belonging to one of the light bands of its spectrum. The principal investigation was concerned with the chloroplasts of *Elodea*, for, with the exception of *Selaginella*, the general structure of chloroplasts was found to be very similar. According to Zirkle they are hollow, flattened ellipsoids, with a central vacuole, which may contain one or more grains of starch. The general granular appearance of the stroma is due to the presence of numerous pores which connect the central vacuole with the cytoplasm surrounding the chloroplast. Around each chloroplast is a more or less permanent sheath of hyaline non-granular cytoplasm. The pigments of the chloroplasts are intimately mixed and evenly distributed throughout the protein-ground substance; a point confirming Wager (1905), but contradicting Priestley and Irving (1907), who considered that the pigments are distributed round the periphery of the chloroplasts. Zirkle found a certain differentiation of chloroplastids; some contained a little starch and were mainly concerned with photosynthesis, and others included much starch functioned mainly as storage bodies.

Zirkle (1927) has also attempted to trace the origin of chloroplasts. In *Lunularia vulgaris* and *Elodea canadensis* he con-

side of them to originate from primordia indistinguishable from mitochondria. In *Lunularia vulgaris* the plastid development was followed from the apical cell through the mature epidermal cells to cells deep within the thallus. The plastids are considered to originate from these primordia in the apical cell. The primordia are not formed *de novo*, but are formed by division; the division taking place in one plane. In character, the primordia are small, colourless bodies, like prolate spheroids, and are soluble in such fixatives as acetic acid, but are rendered insoluble by treatment with alcohol. A similar state of affairs exists in *Elodea*, but the primordia are smaller and often strung end to end, and may frequently be found grouped round the nucleus. They enlarge, develop chlorophyll, and become plastids. The cells immediately surrounding the apical cell first of all become grass-green, and the primordia are no longer soluble in fixatives containing acetic acid.

Andersson (1928) has described a curious situation in a variegated species of the fern *Adiantum cuneatum*. The germination of the spores of this variegated form give rise to two kinds of prothalli, some with normal green chloroplasts and others having small green chloroplasts. The prothalli possessing the small green chloroplasts soon stop developing, and were observed generally to reach a size of two cells, and rarely more than six. In the meanwhile, the chloroplasts became more and more irregular in shape and the green colour seemed to vanish. Even the prothalli with normal chloroplasts developed more or less pale green patches. They were thus variegated like the sporophyte. These pale patches originate at the growing point, and the change in colour is a sudden one, any cell containing chloroplasts of only one kind. Both the green and the pale green plastids contain starch, although in the latter the grains are small. All the green cells contain plastids of the same size, and the same can be said of the cells with the pale green plastids; the two kinds were never found to be mixed. This suggests that the cell in some way controls the character of its contained chloroplasts. There is other evidence to the same effect. As regards the mitochondria, it is known that they are separated according as to

where they happen to lie when the cell wall is formed in cell division, and the long mitochondrial threads may even be cut across by the cell wall. The change affecting the size of the chloroplasts is sudden, but it is possible that the change affecting colour is primary, and that affecting size secondary.

The nature of the segregation visible in the tissues of the gametophyte constitutes a special problem. The green and white areas in the prothalli presumably differ in their genetic capacities, inasmuch as the prothallus is a haploid structure and genetic segregation occurs after meiosis in which the character of the spores is determined.

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## CHAPTER III

### NITROGEN METABOLISM

*Source of Nitrogen for the Plant. The Proteins—Iso-electric Point of Proteins—Hydrolytic Products of the Proteins—Constitution of the Proteins—Classification of the Proteins—Primary Protein Synthesis in the Plant—Secondary Protein Synthesis and Protein Degradation—Nitrogen Metabolism of the Leguminosæ—Function of Urea in the Plant.*

OUR present knowledge of the nitrogen metabolism of plants is still in a very controversial state. A number of recent investigations have, however, extended our knowledge in a variety of different directions.

The plant physiologist has always been hampered in two respects with regard to plant metabolism; firstly, he has had to investigate a number of complex chemical reactions taking place in a single cell, and secondly, the paths along which substances are carried in the plant have never, till recently, been systematically investigated. A further difficulty lies in the fact that the plant, unlike the animal, is a synthetic machine and is able to build up nitrogen compounds of complex chemical constitution from such simple initial products as carbon dioxide, water and nitrates, or ammonium salts, while, in the case of the Leguminosæ, free atmospheric nitrogen forms one source of nitrogen. The whole subject is beset with difficulties, not the least of which is the fact that Abderhalden and his co-workers have recently cast doubts on Hofmeister and Fischer's original conception of the constitution of the proteins.

Ordinary green plants, with a few exceptions, such as the Leguminosæ, obtain their nitrogen from the soil. Persistent claims have been made in recent years that wheat is able to assimilate free nitrogen from the air, but these experiments still remain to be sub-

stantiated. Nitrates and ammonium salts are considered to be the chief sources of nitrogenous food for green plants. Of the two, nitrates are probably the more efficient. Many fungi, both parasites and saprophytes, have to be supplied with organic nitrogen, although some can utilise mineral nitrogen salts, e.g., *Pyronema confluens* and *Pyronema domesticum* (see Claussen, 1912, and Tandy, 1927). Returning to the higher plants, the study of their nitrogen nutrition is complicated by the difficulty of growing them under sterile conditions in which bacterial decompositions are averted. All normal soils contain active nitrifying organisms which can transform ammonia and ammonium salts into nitrates, and care has to be exercised in the interpretation of comparative experiments on the value of ammonia or nitrates as a source of nitrogen.

According to Stewart, Thomas and Horner (1925), pineapple plants are able to assimilate all their nitrogen from ammonium salts, although the best growth is made in nitrate solutions. In the early stages of growth the nitrate culture solutions became more alkaline in reaction, but later more acid than the original cultures. The culture solutions containing ammonium salts showed an acid reaction. A curious fact was discovered in this connection: that plants grown in culture solutions with ammonium salts as a source of nitrogen always contained less calcium than the corresponding nitrate series. It would thus appear that although the pineapple can assimilate ammonium salts it prefers nitrates as a source of nitrogen. Smirnov (1928) grew etiolated barley seedlings in culture solutions containing ammonium chloride, and found an increase of amino-nitrogen, indicating assimilation of ammonia. Calcium increased the assimilation in the preliminary and decreased it in the later stages of the process. The nature of the ammonium salts also has an important influence. Söderbaum (1917) found that ammonium phosphate was more efficient than ammonium sulphate, while ammonium chloride was definitely toxic to some plants. Fred (1924) has shown that when barley was grown in sterile sand culture in which the nitrogen was supplied as ammonium sulphate, the percentage of nitrogen in the crop was very much less than

when nitrifying organisms were present. The consensus of evidence, therefore, seems to point to nitrates being the more efficient nitrogenous food for the higher green plants.

### The Proteins

The most important nitrogenous compounds found in living things are the proteins which form an integral part of protoplasm. It will be necessary here to summarise briefly the more salient chemical and physical properties of these substances for a better understanding of the ensuing discussion on their synthesis by the plant.

The proteins are found in the living portions of all plants. They are present in the dissolved condition in the cell sap, in the semi-dissolved condition in the protoplasm and in the solid state in the storage organs, *e.g.*, seeds, roots, bulbs, tubers, etc. In many cells the undissolved protein occurs in well-defined crystals of different shapes, and also in the amorphous condition. The proteins in seeds are the only ones that have been thoroughly investigated, as they occur in these organs in comparatively large amounts and can be more easily isolated. There is at present little evidence available about the nature of the proteins present in metabolically active cells. A step, however, has now been made in this direction by Chibnall and others (see below).

All proteins contain the elements: carbon, hydrogen, oxygen, nitrogen, sulphur, and sometimes phosphorus and iron are also present. The average composition shows the following values:—

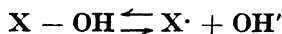
C	=	50.6	to	55.0	per cent.
H	=	6.5	to	7.8	„ „
O	=	21.5	to	23.5	„ „
N	=	15.0	to	19.3	„ „
S	=	0.8	to	2.2	„ „
P	=	0.0	to	0.9	„ „

They are colloids, and can be considerably purified by dialysis. It has been found that complete purification by this method is impossible, and it has so far proved impossible to free any protein from traces of inorganic salts. On keeping, they undergo a

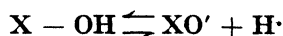
change known as coagulation. Coagulation can also be brought about by heat, enzymes, and the addition of alcohol. Dissolved salts and the pH of the medium are also important factors in this connection. A low pH is most favourable for coagulation, and a high one may prevent it. They are all lævo-rotatory, turning polarised light to the left.

Proteins suffer physical precipitation on the addition of salts to their solutions. No chemical change is produced, and they still retain all their original properties and solubilities. The only proteins not thrown down in this way are the peptones (see below). Proteins give certain characteristic colour reactions with different reagents due to the presence of particular groupings in their molecule, and give precipitates with a number of alkaloidal reagents, such as tannic, phosphotungstic and trichloroacetic acid. They also give insoluble precipitates with the salts of the heavy metals.

**The Iso-electric Point of Proteins.** The proteins behave both as acids and bases ; in other words, they are amphoteric electrolytes. These amphoteric electrolytes or ampholytes play the part of bases in the presence of acids :—



and with bases they function as acids :—



a phenomenon termed electrolytic tautomerism. At a particular hydrogen-ion concentration the number of protein ions and kations will be exactly equal ; this concentration of hydrogen ions is termed the iso-electric point, and the protein will move neither to the anode nor to the cathode, and its solubility at such a point will be at a minimum. The iso-electric point or zone may fall in either an acid or alkaline medium, depending on the relative number of acid or basic groups in the protein molecule. If the acid groups be numerous it will fall in an acid medium, if the basic groups be numerous it will fall in an alkaline medium. Should both be weak, there will be a wide zone of reaction throughout which the protein will be very slightly combined with either acid or base, and as a first approximation, may be regarded as

being free from such combination. It will be seen from this that the iso-electric point does not necessarily coincide with the point of neutrality ( $pH = 7$ ) and varies for different proteins. Pearsall and Ewing (1924a, 1924b) have measured the iso-electric points of a number of plant proteins and have found the following values :—

Edestin . . . . .	$pH = 5.3$ to $5.6$
Legumin . . . . .	„ = $4.4$ „ $4.6$
Globulin (yeast) . . . . .	„ = $4.6$
Globulin (carrot) . . . . .	„ = $4.1$ to $4.4$
Globulin (tomato) . . . . .	„ = $4.0$
Albumin (yeast) . . . . .	„ = $4.6$

Chibnall (1926) has obtained the following figures in a comparison between the iso-electric points of certain leaf cytoplasmic proteins and the hydrogen-ion concentration of the leaf-cell contents.

	Iso-electric point of Protein.	$p-H$ of Cell Contents.
Hogweed . . . . .	$5.0-4.3$	$6.19$
Cabbage . . . . .	$4.7-4.0$	$5.60$
Rhubarb . . . . .	$3.5$	$4.00$
Broad Bean . . . . .	$5.1-4.3$	$5.69$

Examination of these figures would seem to indicate that plant proteins are present in a solution which is on the alkaline side of their iso-electric points ; such proteins therefore function as anions. An exception was found to this rule in the vine in which the iso-electric point of the protein is  $4.8-4.4$  and the  $pH$  of the cell contents  $3.02$ .

It would appear from these results that if the hydrogen-ion concentration of the cell sap were to approach that of the iso-electric point of the cell proteins, the latter would tend to precipitate with serious results to the cells.

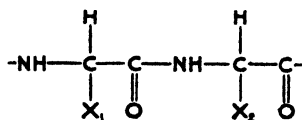
Chibnall and Grover (1926) have investigated the proteins in the cytoplasm of leaf cells. It was found that the protein in the cytoplasm could be separated into two fractions : (i.) combined proteins, which were in some kind of loose combination with substances soluble in alcohol ; and (ii.) soluble proteins, which were uncombined and passed into solution when the cytoplasmic

gel was ground with water. These soluble proteins belonged to the class glutelins and possessed very similar chemical properties. They had an iso-electric range from pH 4.0 to 5.0 in which their solubility was at a minimum. In all cases the leaf cell sap had a higher pH than the range given above, indicating that the proteins were in the form of anions in the cell.

On hydrolysis with acids and enzymes and by fusion with caustic alkalis, the protein molecule is disrupted and gives rise to a number of amino-acids. These amino-acids are bodies in which one or more of the hydrogen atoms (other than carboxyl hydrogen) are replaced by the amino-group  $\text{—NH}_2$ . The simplest of these amino-acids is glycine or amino-acetic acid,  $\text{CH}_2 \cdot \text{NH}_2 \cdot \text{COOH}$ . All the amino-acids, up to the present, that have been isolated from protein hydrolysis belong to the class known as  $\alpha$ -amino-acids, *i.e.*, the amino-grouping is attached to the carbon atom immediately adjacent to the carboxyl group.

### The Constitution of the Proteins

Although the amino-acids are the cleavage products of proteins and are obtained by their hydrolysis, yet the proteins themselves give very little free nitrogen when treated with nitrous acid; a characteristic of the amino-acids which give a quantitative yield of nitrogen when so treated. Arguing from this and other facts Emil Fischer put forward the view that the protein molecule is built up of chains of amino-acids in which the amino-grouping of one acid is combined with the carboxyl group of another :—



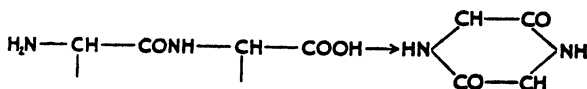
where  $\text{X}_1$  and  $\text{X}_2$  represent different amino-acid radicles.

Such compounds have been synthesised in the laboratory and are known as peptides. An example of a peptide is glycylglycine,  $\text{CH}_2(\text{NH}_2) \cdot \text{CO} \cdot \text{NH} \cdot \text{CH}_2 \cdot \text{COOH}$ . Since this is formed from two amino-acids it is termed a dipeptide. Tripeptides are formed from three amino-acids, and the series can be extended till com-

pounds are reached with many amino-acids in their molecules ; these are called polypeptides. Fischer was able to synthesise a polypeptide with eighteen amino-acid radicles in the molecule, and Abderhalden has obtained one with nineteen such radicles and a molecular weight of 1,200.

These synthetic products show a close resemblance to the natural peptones. The majority are soluble in water, and (with the exception of certain *di*- and *tri*-peptides) give the biuret reaction, a blue colour with alkaline copper sulphate solution. They are precipitated by phosphotungstic acid and have a bitter taste like many natural peptones. They are readily hydrolysed by acids, and they can also in many cases be hydrolysed by proteolytic enzymes. The closest resemblance is found to the proteins in those peptides which have a long chain composed of different amino-acid radicles.

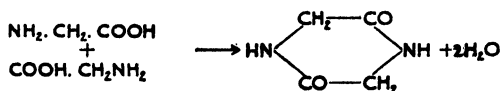
There is no reason to suppose that the amino-acids in the protein molecule are not linked in other ways as well. Fischer himself suggested ring formation of the following type :—



showing the possibility of a piperazine group being present in the molecule, and ring formations of this type are readily accounted for by the elimination of water from a complex of two amino-acid molecules.

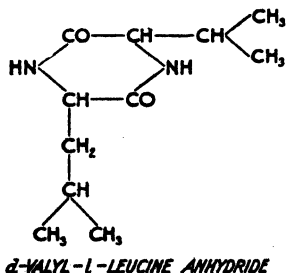
Fischer's views on the constitution of the proteins were unquestioningly accepted for over twenty years and undoubtedly played an enormous part in the furtherance of the study of these complex substances, but recently Abderhalden has introduced a new conception of the constitution of the protein molecule. For a summary of his views the review by Klarmann (1927) should be consulted. The matter can only be briefly dealt with here, and Abderhalden's original papers are too numerous to be given adequate mention in a text of this nature. Abderhalden pointed out the well-known fact that the cleavage products of the proteins by chemical means or by peptic and tryptic enzymes has often

yielded compounds with cyclic and not straight polypeptide chains, *e.g.*, 2 : 5-dioxopiperazines have been found after protein degradation. These 2 : 5-dioxopiperazines are composed of two amino-acids and their simplest representative is glycine anhydride which is formed if an aqueous solution of glycine is left standing for some time :—

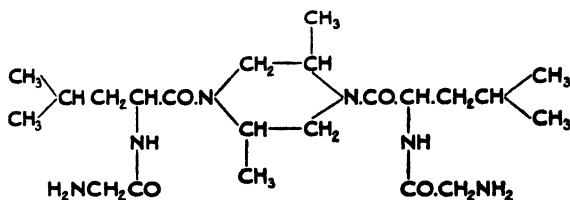


The two amino-acids concerned may be the same or different, and they may also differ in their spacial configuration. Thus a great variety of these cyclic products are possible. Abderhalden regarded the protein molecule as a complex polymeride of these cyclic compounds.

Two views are possible in connection with these substances : (a) they exist as such in the protein molecule ; and (b) their formation is secondary and occurs after the breakdown of the molecule. By partial hydrolysis of the protein, concentration of the product *in vacuo* and subsequent extraction with ethyl acetate, Abderhalden and his workers have been able to isolate a number of new diketopiperazine derivatives. Extreme care must be exercised in these experiments, so that diketopiperazine formation by secondary condensation is excluded. From the chemical standpoint these diketopiperazines are rather resistant towards acids. Abderhalden isolated *D*-valyl-*L*-leucine anhydride by the hydrolysis of casein with 5 or 10 per cent. sulphuric acid and extraction of the dry product :—



Abderhalden contended that it is improbable that this procedure should lead to secondary formation of the anhydride. Further evidence in favour of his views on protein constitution comes from their oxidation products with potassium permanganate and reduction with sodium and alcohol. Both these treatments give oxamide, though in the reduction process the yield is very small. He has also been able to synthesise these substances, and has obtained, for example, diglycyl-leucyl-2 : 5-dimethylpiperazine :—



Diglycyl-leucyl-2 : 5-dimethylpiperazine

But neither this nor any of the synthetic products obtained by Abderhalden were attacked by proteolytic enzymes.

Osborne and Vickery (1928) have strongly criticised Abderhalden's views on protein constitution, and although they admitted that the mass of evidence brought forward by him is impressive, yet they very rightly recalled the fact that without exception all the synthetic products so far obtained have been quite resistant to resolution by enzymes. Fischer's products, on the other hand, were resolved in a number of cases by trypsin, and this fact alone is a strong argument in favour of the retention of the old polypeptide hypothesis, but some recent work on protein synthesis in the potato (see below) appears to support Abderhalden's views on the constitution of the proteins.

### The Classification of the Proteins

At present, there is no very satisfactory classification of the proteins. The best, perhaps, is the scheme of the American Committee on Protein Nomenclature which is given below :—

*I. Simple Proteins.*

- (a) Albumins.
- (b) Globulins.
- (c) Glutelins.
- (d) Prolamines.
- (e) Albuminoids.
- (f) Histones.
- (g) Protamines.

*II. Conjugated Proteins.*

- (a) Nucleoproteins.
- (b) Glycoproteins.
- (c) Phosphoproteins.
- (d) Hæmoglobins.
- (e) Lecithoproteins.

*III. Derived Proteins.**(i.) Primary protein derivatives.*

- (a) Proteans.
- (b) Metaproteins.
- (c) Coagulated proteins.

*(ii.) Secondary protein derivatives.*

- (a) Proteoses.
- (b) Peptones.
- (c) Peptides.

Of these proteins, the glutelins and prolamines are only represented in the vegetable kingdom and the albuminoids and protamines are characteristic products of animals.

Only the properties of the larger groups will be considered here as far as these bear on the discussion of protein synthesis in the plant. For a full description of the properties of the vegetable proteins the reader should consult the monograph by Osborne (1924).

**Albumins.** These are not very abundant in plants. They are readily soluble in water and coagulated by heat. They are not precipitated by sodium chloride or magnesium chloride, but are precipitated by a saturated solution of ammonium chloride. The best characterised vegetable albumins are leucosin in the grain of cereals, legumelin in soya-bean, cowpea, and lentil, phaselin in the kidney bean and ricin in the castor bean.

**Globulins.** These are proteins insoluble in water, but soluble in weak saline solutions. They are readily coagulated by heat, and precipitated from solution by magnesium sulphate and half-saturated ammonium sulphate.

**Glutelins.** This group includes those proteins which are not dissolved by neutral aqueous solutions, saline solutions, or alcohol. They are soluble in weak alkali or acid. Glutelin occurs in wheat and oryzenin in rice.

**Prolamines.** This is a group of proteins characterised by their solubility in 70 to 90 per cent. alcohol. On hydrolysis they yield large amounts of glutamic acid, proline and ammonia, small amounts of arginine, histidine and little or no lysine.

Certain derivatives of the proteins can also be considered here. The metaproteins, for example, obtained by the hydrolysis of protein by trypsin or pepsin. The metaprotein obtained is termed acid or alkali albumin, depending on whether pepsin or trypsin is used for the hydrolysis, since the former works in alkaline, and the latter in acid media.

In general terms it can be said that similar proteins are found only in seeds of the same natural family. This has an important influence on the subsequent development of the embryo, which in the first stages of germination obtains its nitrogenous food by the hydrolysis of the reserve proteins present in it. These proteins are among the final products of the metabolism of the plant, and, in the primary stages of development, the embryo is supplied with a definite food, which for each member of the same species is the same. Each member of a species thus begins its individual life under similar chemical conditions which are different for those of any other species. It would thus appear that by the time the green plant has reached the stage of development when it obtains its food from the soil and air, its chemical processes have already been established along certain definite lines which it must follow for the rest of its life-cycle.

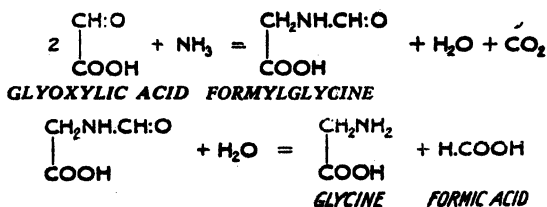
### Primary Protein Synthesis in the Plant

The problem of protein synthesis in the plant has naturally attracted greater attention than the synthesis of any of the other

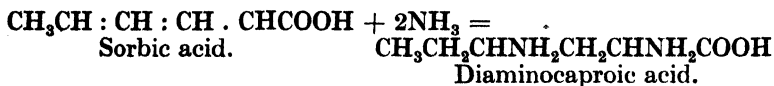
nitrogen fractions. The evidence for the various steps in the process and the possible seat, or seats of synthesis, have all been investigated from time to time, but the accumulated evidence is both confusing and conflicting, and it is difficult to present the subject as a coherent whole.

Before considering the possible places in which protein synthesis may take place, it will perhaps clarify the ensuing discussion if the various methods whereby protein is built up are considered first. A distinction should be kept in mind between *primary* protein synthesis in which protein is built up from simple inorganic nitrogen, and *secondary* protein synthesis in which protein is first broken down to simpler bodies and translocated from the centre of synthesis to other parts of the plant and reformed into protein once more. With regard to primary protein synthesis, on one view, there is individual synthesis of each amino-acid, and the amino-acids are subsequently condensed to give peptides, peptones, proteoses and proteins. The second view of synthesis is concerned with Abderhalden's ideas on the constitution of the protein molecule. According to this view separate synthesis of each amino-acid does not take place, but large constituent molecules of protein are laid down by condensation from compounds of greater simplicity.

On the first view of protein synthesis, *i.e.*, by separate synthesis of each constituent amino-acid, there is (1) the consideration of the introduction of the amino-group ( $\text{NH}_2$ ), and (2) the condensation of amino-acids through peptides, peptones, etc., to ultimate protein. The synthesis of amino-acids has been brought about in the laboratory by the direct action of ammonia on aliphatic and aromatic acids. Erlenmeyer and Kunlin were able to synthesise formylglycine by the action of ammonia on glyoxylic acid :—



The formylglycine is readily hydrolysed to glycine and formic acid. Again, Fischer and Schlotterbeck synthesised diamino-caproic acid by the interaction between ammonia and sorbic acid, an unsaturated acid present in the unripe berries of the mountain ash :—



It should be noted that these products are  $\alpha$ -amino-acids. Nevertheless, it is a far cry from such laboratory reactions to the synthesis of these acids by plants, and it is in the highest degree unsafe to draw analogies between test-tube reactions and the chemical reactions of the living cell. The problem of amino-acid formation from nitrates will be considered later.

The second stage in the process whereby amino-acids are condensed to give rise ultimately to protein may be brought about by the action of proteolytic enzymes, which may catalyse the synthetic side of the equilibrium.

Passing now to a consideration of the second view of protein synthesis, *i.e.*, that this is brought about by condensation *en bloc* of simpler products than amino-acids to give complex protein, this theory is in part based on the function of the amide, asparagine,  $\text{COOH} . \text{CH}(\text{NH}_2) . \text{CH}_2 . \text{CONH}_2$ , in the nitrogenous metabolism of the plant. Asparagine bulks largely in the literature of plant nitrogen metabolism and various investigators have invested it with a number of diverse *rôles*, and like King Charles' head to Mr. Dick, asparagine sooner or later becomes involved in any discussion of this subject.

As far as protein synthesis is concerned, if asparagine is the primary stable product of nitrogenous metabolism, as some have assumed, and is supplied to the tissues in which protein synthesis is taking place, together with a supply of carbohydrates, then, by respiration, it could give rise to two-carbon residues and ammonia. These, together with other active molecules, such as pyruvic aldehyde and acetaldehyde, might condense *en bloc* to give large polypeptide complexes. It is, however, very doubtful if this be

the case. Maskell and Mason (see Chapter V) have shown that in the cotton plant asparagine apparently functions as a storage product, while Prianischnikow in a long series of investigations (see below) considers that the rôle of asparagine is to combine with free ammonia which is toxic to living tissues if allowed to accumulate. The present author and his colleague, Mr. Alan M'Bain, in their investigations on the nitrogen metabolism of the potato were able to show that there is a particularly close relationship between ammonia and asparagine.

The question now arises: What is the centre of primary protein synthesis? There seems to be little doubt that the leaf is one centre and possibly the chief centre of primary protein synthesis, but the problem here is complicated by protein hydrolysis and translocation. There is also evidence available at present that synthesis can take place in the roots of certain plants. W. Thomas (1927) found reduction of nitrate in the finer roots of apple trees, while Nightingale and Schermerhorn (1928) also found reduction of nitrate to take place in the fibrous roots of asparagus, and elaboration of organic nitrogen appears to take place in this region. In the case of seedlings the problem is in some degree simpler. In the early stages of development there is breakdown of reserve material and resynthesis in some other part of the newly-developing plant. The case of the leaf will be considered here immediately, and the nitrogen metabolism of the seedling discussed in the section on Secondary Protein Synthesis and Protein Degradation.

**The Leaf.** Nitrate enters the leaf in the transpiration current and presumably forms the initial substance for the complex series of changes that leads to ultimate protein synthesis. That there is a diurnal variation in nitrogen in leaves has been established by various workers. Chibnall (1924) showed that there is an increase by day and a decrease by night of protein in the leaves of *Phaseolus multiflorus*. An increase in total nitrogen by day and decrease by night has been found for the leaf of the cotton plant by Maskell and Mason (see Chapter V), and by Cockerham (1933), and also by Barton-Wright and M'Bain (1933) for the potato leaf. It is evident, then, that in the leaf there must be

synthesis by day, and presumably hydrolysis and translocation out of the leaf blade by night.

In 1890 Sapochnikow established the fact that there was an increase of protein with increase of carbohydrate in the leaf in the presence of light. It has also been discovered that nitrates accumulate in leaves in the dark and disappear in the light. In variegated leaves it is only from the green portions that nitrates disappear. It was therefore concluded that leaves were the chief centre of protein synthesis and that the process could only take place in the light.

Light, however, is not apparently a necessary factor. In 1901 Zalesski found that protein synthesis could occur in leaves in the dark in the presence of nitrates and carbohydrates. The necessary factor for the synthesis appeared to be carbohydrate and not light. The function of the light was to give to the leaves the power of photosynthesis, and thus only indirectly helped in the synthesis of proteins. M. E. R. Robinson (1929) has criticised this work on the grounds of technique. The protein was estimated by the method of Stutzen, but amino-acids and carbohydrates can combine at low temperatures to give complexes precipitable with copper sulphate, so that the increase registered may have been due to this cause and not to true protein nitrogen; but the recent investigations of Muenscher (1923) have more firmly established the fact that protein synthesis can take place in the dark and that light is not a necessary factor. He grew *Chlorella* in nutrient solutions containing nitrogen, either as calcium nitrate or ammonium sulphate in diffuse light, and also in total darkness for 105 to 235 days. Quantitative determinations were made of the volume, dry-weight and total nitrogen, and these showed strong evidence that the alga could synthesise protein in the absence of light when nitrogen was supplied in the form of inorganic salts.

It has already been stated that nitrates form the starting-point of protein synthesis; but nitrates, chemically speaking, are simple bodies, whereas the proteins are complex bodies of high molecular weight. Adair (1924) has calculated that albumins have a molecular weight of 68,000 and globulins a still higher

value, 150,000. It is obvious, therefore, that the process must take place in stages.

Although light does not appear to be an essential factor for protein synthesis, the presence of potassium seems to be necessary. Beet, for example, forms less protein in the absence of potassium. Although protein nitrogen is less in such circumstances, it has been shown by Burrell (1926) that there is an accumulation of amino-acids. Calcium is also an important factor, and in the absence of this element there is a considerable accumulation of nitrate in the cell. (See section on Calcium in Chapter IV.)

In connection with the question of nitrates and their relation to protein synthesis, it must be remembered that the nitrates are, relatively speaking, stable bodies, and comparatively inert. They must therefore be brought into a more reactive condition. The probabilities are that the first stage of the synthesis of protein in the living cell is the conversion of nitrate into nitrite. This was first demonstrated in 1890 by Laurent and later confirmed by Irving and Hankinson (1908) for the leaves of *Sagittaria*. Anderson (1924) found nitrate to be present in the shoots of about forty different species of plants as well as in the seeds of *Brassica nigra*, *Cannabis sativa* and *Lepidium sativum*. The amount of nitrate in the plants appeared to vary with the season. Thus in *Mercurialis perennis*, positive reactions were obtained in October and negative in June, whereas material tested for nitrite in October gave negative results and positive results were obtained in June, i.e., the reverse of the nitrate. The presence of nitrate in the plant, according to Anderson, appears to depend to a considerable extent on the richness of the soil. Thus, she found that plants growing in highly manured soil gave a strong positive reaction, possibly because they had absorbed more nitrate than they could elaborate in a given time.

Anderson showed that there is some kind of nitrate-reducing mechanism in the plant by the following experiment: 10 drops of a 4 per cent. solution of sodium nitrate was added to 10 c.c. of expressed sap, and the liquid was then equally divided between three test-tubes. To one of the tubes was added 2 drops of

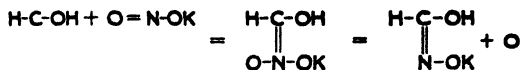
acetaldehyde (10 per cent.), and all three tubes were then placed in a water bath at 45° C. The tube without the aldehyde was removed at the end of ten minutes, and the other two at the end of twenty minutes. After cooling, the extract was half saturated with ammonium sulphate, filtered to remove chlorophyll, and tested for nitrite with the Greiss-Ilosvay reagent. The tube with acetaldehyde gave a positive reaction. The actual substance responsible does not appear to be an enzyme, but is of the nature of the oxidisable material "atite" discovered by P. Haas and Hill (1923) in milk, which reduces nitrate to nitrite. Anderson considered it doubtful if this substance plays any part in protein synthesis, since it is only under artificial conditions of high temperature (45° C.) and in the presence of aldehyde that the reduction takes place. It is unfortunate that this work was never carried any further and the influence of iron investigated on the reducing mechanism, for Eckerson (1924) has shown that the juice extracted from various parts of the tomato possesses the power of reducing nitrates to nitrites and finally gives ammonia. This process can occur in either light or darkness and boiled extracts will also bring about the change, provided that the pH of the medium is kept at 7.6. It was discovered that the important factor in this reducing process was the presence of iron.

It has been known for a considerable time that nitrates can be reduced to nitrites photochemically, *i.e.*, under the influence of light, but this change will not occur in the dark. The situation is therefore a curious one: protein synthesis can take place in the dark provided that carbohydrates are present, yet nitrites are produced from nitrates photochemically. Eckerson's investigation, however, goes a long way to show that the plant possesses some mechanism whereby nitrates can be reduced to nitrite and even ammonia in the absence of light, and light therefore does not enter as a factor into protein synthesis.

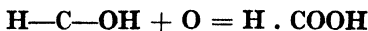
Various schemes have been put forward to show the steps whereby nitrate is converted to amino-acids. For example, Baly, Heilbron and Hudson (1922), and Baly, Heilbron and Stern (1923) assumed that protein synthesis is a photochemical process, and claimed that the first stage in the reaction is the

production of active aldehyde from carbon dioxide and water, and nitrite from nitrate under the influence of light.

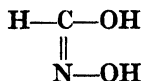
The nitrite and active aldehyde then combine to give formhydroxamic acid as the potassium salt, one atom of oxygen being split off in the course of the reaction :—



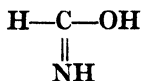
The oxygen liberated is said to oxidise some of the active aldehyde to formic acid :—



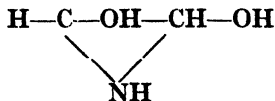
Under the experimental conditions used by these investigators, the formhydroxamic acid was alleged to be completely hydrolysed to the free acid :—



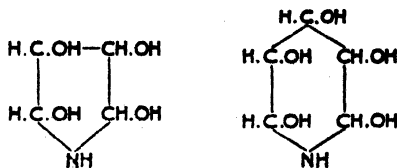
This compound was considered to lose oxygen to give :—



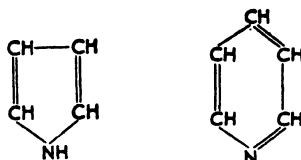
Such a substance could condense with more active aldehyde to yield first :—



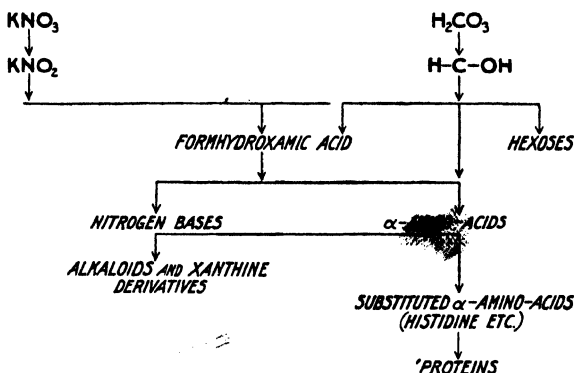
and this by intramolecular change would give glycine,  $\text{CH}_2\text{NH}_2\text{COOH}$ . It could also condense with either three or four molecules of active aldehyde to give cyclic derivatives :—



these, by loss of oxygen and water, would give pyrrole and pyridine derivatives :—



Baly, Heilbron and Hudson have summarised their results in the following scheme :—

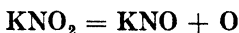


These reactions are all based upon *in vitro* experiments. Baly and his co-workers presupposed light to be an essential factor. It has already been seen that light is not a necessary factor, and it is the presence or absence of carbohydrates that is necessary. In any case, this work has been submitted to such drastic criticism both in this country and in America, that little reliance can be placed upon it.

Schimper showed many years ago that the reduction of nitrates to nitrites in leaves was in some way connected with the presence of iron compounds and not with light since chlorotic leaves with no iron failed to reduce nitrates. It has since been shown by Baudisch (1921, 1928) that the cholera bacillus in peptone culture has the power of accumulating iron within it in large amounts, and it also possesses the power of reducing nitrates

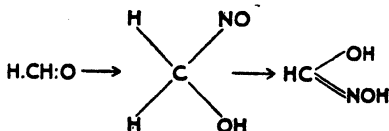
to nitrites to an altogether remarkable extent. Moreover, it was discovered that this reduction was directly correlated with the iron content and oxygen respiration. Baudisch has also demonstrated that one of the oxygen atoms in the nitrate molecule is differently combined to the others and very much more readily split off, either under the influence of light or iron to give nitrite, the reactions being fundamentally the same and the iron functioning as a catalyst.

Baudisch found that aqueous solutions of potassium nitrite containing easily oxidisable substances such as ethyl alcohol, aldehydes, sugars, starches, etc., suffer a comparatively rapid reduction under the influence of diffused daylight, the nitrite being converted into potassium nitrosyl and oxygen :—

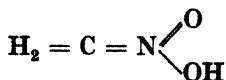


Complex iron salts were also found to bring about the reaction. Thus, in the presence of a trace of iron, a faintly alkaline solution of glucose and potassium nitrite gave potassium nitrosyl and ammonia; but nitrates could not be reduced in this manner. Apparently the reduction of nitrate in biological reactions needs the presence of oxygen, and it has long been known that ferrous salts in the presence of oxygen instantly reduce nitrates to nitrites.

Nitrosyl itself readily reacts with formaldehyde to give form-hydroxamic acid, with the intermediate production of nitroso-methyl alcohol. The latter, by intramolecular rearrangement, gives the formhydroxamic acid :—

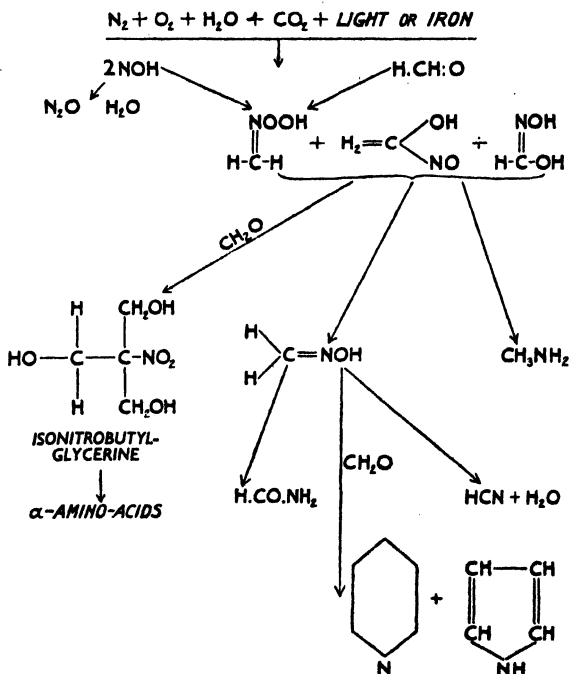


Formhydroxamic acid is quite stable in the dark, but in the presence of light changes over partly to aci-nitroso-methane :—



Longer exposure to light yields methylamine,  $\text{CH}_3\text{NH}_2$ .

Baudisch, by means of colour reactions, discovered that under the influence of light the formhydroxamic acid gives formaldoxime, and a second portion gives hydrocyanic acid and water. The presence of the oxime was found in peptone cultures of the cholera bacillus. The anhydrous product rapidly polymerises to the tri-molecular form. In weakly alkaline solution and in light it undergoes intramolecular change with the formation of an intermediate labile compound which is capable of linking itself with more formaldehyde with the formation of complex nitrogen cyclic derivatives. Summarising these results we have :



It is possible, as Baudisch suggested, that under the influence of nitrosyl, amino-nitrogen is introduced into the higher alcohols, sugars, etc., to give amino-acids, since it is so easily able to enter into different combinations, and that the amino-acids are subsequently condensed to give the proteins.

Thus far we have considered the formation of amino-acids from nitrates. There is, however, another possibility that must also be considered here, namely, whether asparagine is the chief source for protein synthesis. Such a view has been expressed by Chibnall and supported by McKie (1931). McKie working with lupin found that over a growing season of eighty-one days, protein, insoluble nitrogen compounds, and asparagine, represent by far the largest components of the total nitrogen. The most conspicuous feature of McKie's curves was the behaviour of asparagine, which increased rapidly to a maximum and then fell away, and with this fall in asparagine there was a corresponding increase in protein.

Barton-Wright and M'Bain (1933) are not in agreement with the view that asparagine plays an all-important part in protein synthesis as far as the potato is concerned. These investigators have determined total nitrogen, ammonia nitrogen, asparagine nitrogen, amino-acid nitrogen, nitrate nitrogen, residual nitrogen<sup>1</sup> and protein nitrogen in the laminae and petioles of different potato varieties over twenty-four periods at different times in the growing season. The varieties of potato used in this work were Arran Victory and President. The intention of the investigation was to compare differences, if any, in the nitrogen metabolism of healthy potato plants and plants affected with the virus disease known as leaf-roll. Special virus-free units were therefore used for this investigation and the whole of the material, healthy and diseased, was grown in insect-proof greenhouses. Samples of laminae and petioles were taken at two-hourly intervals and the results expressed as a percentage of the "residual dry weight," *i.e.*, dry weight less total carbohydrates, which gives a more constant basis of expression than the total dry weight (see Chapter V). Each experimental run was commenced at 11 p.m., and completed at 9 p.m., G.M.T. The data were statistically examined and the various correlation coefficients and regression equations calculated.

It was shown that there is a well-marked diurnal variation in

<sup>1</sup> Residual nitrogen was taken as the fraction of the total soluble nitrogen not accounted for by the sum of the ammonia N, asparagine N, amino-acid N, and nitrate N. The nature of this fraction was not determined.

total nitrogen in the laminæ of the healthy leaves, total nitrogen increasing by day and diminishing by night. This was interpreted as showing synthesis by day and hydrolysis and transport by night. With regard to the various nitrogen fractions in the leaf blade, nitrate N was found to accumulate at night and to fall during the day in both varieties. Ammonia N showed a rise in the early part of the day and then fell away, whereas asparagine N in Arran Victory showed, on the whole, a rise during the day and a fall at night. In President, however, it rose sharply to a maximum and then fell away, and the curve for ammonia N and asparagine N were similar in shape and the latter lagged a period of two hours behind the former. Amino-acid content in both varieties was small, and fluctuated during the day, the day values being as a rule higher than the night values. On the whole protein N was found to show a maximum in the early part of the experimental period and also a maximum in the afternoon, while residual N was at a maximum in the morning, and also at the close of the experimental period.

No statistically significant relationship could be discovered between protein N and either asparagine N or amino-acid N, and no significant statistical relationship could be discovered between nitrate N and either ammonia N, asparagine N or amino-acid N, but a particularly close relationship was found between ammonia N and asparagine N, and the regression equation showed that the reaction between these two fractions proceeds in the direction ammonia N  $\longrightarrow$  asparagine N. A reversible reaction was found between asparagine N and amino-acid N, while the relationship between ammonia N and amino-acid N was in the direction ammonia N  $\longrightarrow$  amino-acid N. These reactions were found to take place in both varieties.

Turning now to the question of protein N and residual N formation, it has already been stated that no significant statistical relationship could be found between protein N and the fractions asparagine N and amino-acid N. The direct correlation coefficients between protein N and nitrate N and residual N, however, were found to be statistically significant. Taking for example, the case of Arran Victory, the direct correlations between protein

N and nitrate N and residual N and nitrate N were  $r = -0.5973$  and  $r = -0.7145$  respectively, and both values are fully significant. It was further found that in the healthy plants of either Arran Victory or President, there was apparently no inter-conversion of residual to protein N. The regressions are given below for Arran Victory. P represents protein N, R residual N and D nitrate N.

$$R = .9114 - 1.371D - .0374P \quad . \quad . \quad . \quad (1)$$

$$P = 6.488 - 3.313D - .3369R \quad . \quad . \quad . \quad (2)$$

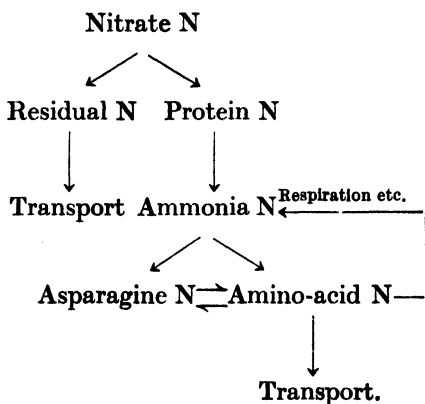
It is clear from equations (1) and (2) that decrease of nitrate N leads to increase of protein N and residual N, whereas inter-conversion of protein N and residual N is not statistically significant.

Moreover, it was found that the reaction between nitrate N and protein N, as well as that between residual N and nitrate N, was independent of other factors. Thus, the partial correlation coefficients for protein N, residual N and nitrate N, in which one or more factors were eliminated, all showed an increase over the direct coefficients.

It was also found that there is a particularly close relationship between protein N and ammonia N and the direction of the reaction is protein N  $\longrightarrow$  ammonia N.

A seasonal examination of the various nitrogen fractions described above was also made in the laminæ at weekly intervals. It was found in both varieties that there was a marked increase in protein N during the first three weeks of growth, while there was a heavy fall in nitrate N over the same period. Both protein N and nitrate N then remained constant in amount over the rest of the growth period. With regard to residual N, this in Arran Victory remained very constant over the whole of the growth period, whereas in President, after an initial increase, it fell away very regularly throughout the whole season of growth.

Barton-Wright and M'Bain put forward the following tentative scheme to account for the metabolism of the healthy potato plant :



It has often been suggested that leaf-roll is a disease of deranged protein metabolism (see Schweizer, 1928, 1980), but Barton-Wright and M'Bain were unable to substantiate this statement. The course of the reactions in leaf-roll Arran Victory and leaf-roll President followed a similar course to the healthy plants. The curves for the various nitrogen fractions were identical, with one or two minor exceptions. There was, however, the difference that in the leaf-roll plants they showed a very regular lag period of two hours between reaching the same maximum and minimum points as in the healthy. In leaf-roll plants it was also discovered that nitrate N passes directly to both protein N and residual N, but at the same time there is interconversion of residual N and protein N. The regressions for President leaf-roll are given below :—

$$R = 4.801 - 1.171P - 1.949D \quad . \quad . \quad . \quad (1)$$

$$P = 3.523 - .8017R - 1.582D \quad . \quad . \quad . \quad (2)$$

Here it is obvious from the equations that unlike the healthy material, interconversion of residual and protein N can take place in the laminæ of the diseased plants.

The seasonal examination of the leaf-roll laminæ showed, as in the healthy, a marked increase in protein N in the early stages of

growth and a marked fall in nitrate N over the same period. A difference, however, was shown in the behaviour of residual N. This fraction, instead of remaining more or less constant as was found in healthy Arran Victory or decreasing as in healthy President, rose very regularly to a maximum at the end of the growing season in the diseased plants of both varieties. Leaf-roll is a virus disease in which the phloem suffers necrosis, and in the circumstances, the main channel of transport out of the leaf is barred. It has been suggested by Maskell and Mason (see Chapter V) that residual N forms the necessary head in the leaf blade for nitrogen transport, and this suggestion would appear to be borne out by the results described above. In the diseased plants the breakdown of the channel of transport leads to accumulation of residual N. Moreover, further support is given for this view by the fact that in President, which is a variety particularly intolerant of the disease and is very severely affected by it, no residual N could be found in the diseased petioles, and the sum of the ammonia N, amino-acid N, asparagine N and nitrate N, was equal to the sum of the total soluble N.

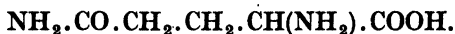
It was suggested by Barton-Wright and M'Bain that nitrates are first reduced to nitrites in the leaf blade and that possibly some such body as formhydroxamic acid is formed, which would by intramolecular rearrangement and condensation *en bloc* lead to the formation of residual N and protein N. The whole difficulty here is that up to the present time, in spite of repeated investigations, the presence of formaldehyde in normal leaves has not been shown with any degree of certainty.

The nature of the fraction or fractions composing residual N are at present quite unknown. McKie (1981) from her investigations of the protein metabolism of lupin seedlings was able to obtain from 84 to 99 per cent. recovery of the total soluble nitrogen when she estimated proteose nitrogen. It is therefore possible that the residual N fraction is mainly composed of proteoses. Certainly, from the results described above, it would appear that this fraction is closely related chemically to protein. The question of ammonia formation will be discussed in the next section.

### Secondary Protein Synthesis and Protein Degradation

Secondary protein synthesis and protein degradation has to be considered from several standpoints. There is in the first place the fate of the reserve protein in the endosperm of the seed, which must be rendered soluble by preliminary hydrolysis and then be removed to the regions of active development. Secondly, there is the fate of the protein elaborated in the leaf, which must be evacuated for storage to other parts of the plants, and lastly there is the fate of the nitrogen transported to seeds where it is resynthesised to reserve protein.

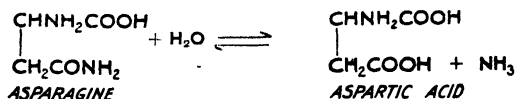
**The Nitrogen Relationships of the Germinating Seed.** In the early stages of germination reserve protein in either endosperm or embryo of the seed is hydrolysed to presumably amino-acids, and these are then translocated to the actively growing regions of development and resynthesised to protein once more. In the Leguminosæ, it is an undoubted fact that asparagine soon makes its appearance on germination and accumulates to a marked extent, and further, far more asparagine is formed than can be accounted for by hydrolysis of reserve protein. The pioneer investigations of Schulze and his co-workers which extended from 1878 to 1910 established the fact that in the Leguminosæ, the aspartic acid present in the reserve protein of the seedling would not account for the great accumulation of asparagine. This was found to be especially the case with etiolated seedlings, for in normal seedlings there is less accumulation, but nevertheless considerable amounts are still present. In other natural families, *e.g.*, the Cruciferae, a similar phenomenon was encountered, but the accumulation product here proved to be another amide, glutamine



According to Schulze, hydrolysis of reserve protein leads to the formation of a mixture of nitrogenous substances which are later converted to asparagine or glutamine and nitrogen is translocated in this form to the newly developing points of the plant and there regenerated into protein once more.

Unfortunately, the reactions whereby protein and asparagine are

interconverted are still very far from clear. The degradation of proteins through proteoses, polypeptides and amino-acids is well known, but there is little evidence regarding the steps between amino-acids and asparagine. It has usually been assumed that the immediate precursor of asparagine is aspartic acid, and that an enzyme, asparaginase, controls the reaction :—



The presence of free aspartic acid in the plant has not been demonstrated, although Kiessel (1924) has put forward a claim that he has isolated this amino-acid from the ears of ripening rye. As no asparagine was discovered even in the later stages of ripening, he considered that aspartic acid could not be the forerunner of asparagine, and that de-amidation of the asparagine must take place at a stage earlier than those he investigated. Grover and Chibnall (1927) pointed out that investigators have failed to differentiate between de-amination and de-amidation, and that the evidence of the existence of an enzyme asparaginase is very meagre and uncertain. They have isolated an enzyme from the rootlets of germinating barley that is capable of liberating ammonia from asparagine. A preparation was made by grinding up the dried rootlets to a fine powder and making this into a thick cream with water. Such a preparation was found to release ammonia from asparagine at a pH of 7.5. The enzyme itself was isolated by precipitation from an aqueous solution with alcohol.

This enzyme is specific in nature and will only attack *l*-asparagine and not the *d*-form to give aspartic acid. It will also attack *d*-glutamine, which is closely related to asparagine. Other amides, such as acetamide and propionamide, were not hydrolysed. The enzyme also attacks glycylglycine with liberation of ammonia. There is thus no question that an enzyme exists in the higher plants capable of effecting de-amidation of asparagine. By adopting the view that the amide group of asparagine is essentially

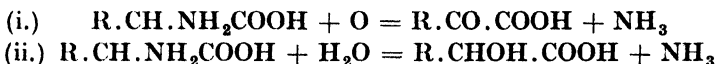
a peptide linkage and that both asparagine and glycylglycine are hydrolysed by a barley peptidase, our conception of protein metabolism, according to Grover and Chibnall, is considerably simplified.

Prianischnikow (1924a, 1924b, 1926) is at variance with Schulze on the question of the function of asparagine. He was able to confirm Schulze's work that asparagine accumulates at germination, but he held the view that it is not readily used for protein resynthesis; moreover, it increases rather than disappears in the presence of carbohydrate, which is a point against its ready utilisation in protein synthesis. According to Prianischnikow, asparagine is formed as a temporary nitrogen reserve whenever there occurs a marked accumulation of ammonia which is toxic to the living cell. This ammonia is rendered harmless by condensation with aspartic acid to form asparagine, and the latter functions much as urea functions in the animal economy. This question of asparagine formation and function in the plant will be further considered below in relation to other plant organs.

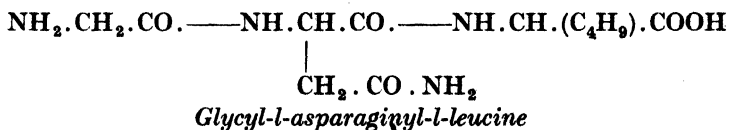
**The Nitrogen Relationships of Leaf and Shoot.** It has already been stated that several investigators have shown that there is a diurnal variation in nitrogen in the leaf, with increase during the day and decrease during the night. According to Chibnall (1922, 1924) from determinations based on wet weight measurements, there is a well-marked diurnal variation in the nitrogen content of the leaves of *Phaseolus multiflorus*, and he concluded that there is a continuous decomposition of protein by day, as well as by night, in the leaf, with a simultaneous withdrawal of the soluble products of this decomposition to other parts of the plant. The process is masked during daylight because the amount of synthesis is greater than that of hydrolysis. Chibnall has put forward the view that there is a continuous production of asparagine from protein in the normal mature leaf, and that in plant metabolism asparagine plays the rôle of a translocatory substance and is the chief medium whereby nitrogen, in a form suitable for subsequent resynthesis, can be conveyed from one part of the plant to another. This view, however, is not acceptable in the light of recent investigations. It has been shown by Maskell

and Mason (see Chapter V) that in the cotton plant marked, negative gradients of asparagine occur in the stem, and this body appears to act more in a storage capacity than a translocatory product, and indeed, it is difficult to see how, in the light of this work, asparagine can act as a translocatory body.

Prianischnikow's views on the inter-relationships of asparagine and ammonia have already been considered above. Barton-Wright and M'Bain are in agreement with his ideas. These investigators showed that the relationship between ammonia and asparagine is particularly close. They were likewise able to show that the relationship between protein and ammonia is also close. In the potato, asparagine does not occur to such an extent among the various nitrogen fractions, and in point of fact in the variety President is very small in amount. With regard to ammonia formation, Barton-Wright and M'Bain were able to discover two sources of formation: (1) from amino-acids and (2) from protein. Ammonia formation from amino-acids can take place in several ways, such as direct oxidation or hydration:



and in the potato a mechanism is known whereby de-amination of amino-acids is brought about by the catechol-oxidase system. Ammonia can also be formed from protein. For example, tripeptides of the type of glycyl-*l*-asparaginy-*l*-leucine



yield, on hydrolysis, free ammonia, and since many proteins yield a certain amount of free ammonia when hydrolysed, this would seem to suggest the presence of acid-amide linkages in the protein molecule. It was also ascertained by these workers that increase or decrease of ammonia can lead to increase or decrease of amino-acids, so that there is presumably amination of organic

acids to amino-acids and de-amination of amino-acids to ammonia and organic acids.

The views of Ruhland and Wetzel (1926, 1927, 1929) can be conveniently discussed at this stage. This work is in many ways an extension of that of Prianischnikow. It will be remembered that Prianischnikow suggested that the function of asparagine was to bind toxic ammonia, which, if allowed to accumulate, would result in the death of the plant. It was pointed out by Ruhland and Wetzel that in plants with very acid sap these conditions do not hold, and accumulation of ammonia in the form of ammonium salts can occur relatively to an enormous extent. To take a concrete example, in *Begonia semperflorens* the sap acidity is high (pH 1.54–1.56). In "normal" plants the acidity varies between pH 5–6.5. When *B. semperflorens* was placed in the dark the respiratory quotient rose from an initial value of 1.1 to 1.47–1.85, whereas in the ordinary plant the quotient falls below 1. It was found that the leaves of this plant contained on an average 20 per cent. of oxalic acid (per cent. dry weight). In the morning the leaves contained 5–10 times as much ammonia (calculated as percentage of total nitrogen) as plants which have no significant amount of organic acids, and this value increased some three-fold during the day. The amino-acid content was shown to be relatively low. Whereas in normal plants the value :

$$\frac{\text{Soluble N} - \text{Ammonia N}}{\text{Ammonia N}}$$

is about 50, in *B. semperflorens* it is only 2–3.

If the leaves were kept in the dark at a temperature of 28–35° C. to bring about protein degradation, the ammonia content rose enormously. Thus, in 106 hours the ammonia rose to 80 per cent. of the total nitrogen, while the initial amount of amide nitrogen present disappeared. De-amination was accompanied by a parallel increase in oxalic acid (the pH of the sap decreased by 1.8) so that the ammonia was always present as ammonium oxalate, and at no time was in the free condition, and ammonia poisoning did not take place. In normal plants, amide nitrogen increases in the dark, ammonia remaining more or less stationary. Ruhland

and Wetzel suggested that plants can be divided into two physiological types: "Amide" plants, which do not have a high sap acidity, and "Ammonia" or "Acid" plants. In the latter type de-amination results in the simultaneous formation of organic acids, e.g., oxalic acid, malic acid, and ammonia, and the latter is prevented from exercising its toxic properties by being bound to the organic acid as the ammonium salt.

Ruhland and Wetzel (1929) have also investigated the nitrogen relationships of rhubarb (*Rheum hybridum*, Hort.). The rhizome of this plant has only a slight acid reaction and contains amides (20 per cent. calculated as per cent. of total soluble nitrogen), much amino-acid (50 per cent. of total nitrogen) and only traces of ammonia. It was ascertained that the petioles of very young leaves are able to synthesise protein rapidly from amino-acids. This synthesis ceases when the leaves open, and later, when the petioles are 15 cm. long, strong de-amination sets in. The fully grown leaf stalks now contain some 62 per cent. of their total nitrogen as ammonia, and if they be kept in the dark this value may rise to as much as 72 per cent. Parallel with this increase in ammonia during growth there is increase in organic acid. The chief organic acid present is malic acid, but a small amount of succinic acid is also found, and these in turn slowly give place to oxalic acid as the petiole ages, so that ammonia toxicity is again prevented. Both *l*-malic and *dl*-malic acid were found to be present, and it was thought by Ruhland and Wetzel that the inactive acid is translocated from the rhizome in which this modification is only to be found, and that the active *l*-malic acid arises by de-amination of amino-acids, since the relative concentrations of ammonia and *l*-malic acid were found to be nearly 1 : 1.

Mothes (1926) claimed to show that protein synthesis is more active in young leaves than in old, a view which is probably correct, as will have been seen from the discussion on protein synthesis. In mature leaves he considered that protein hydrolysis plays the major rôle, and Mothes concluded from his results that asparagine production is a secondary process due to oxidation and has nothing to do with translocation, and he is at one with

in Prianischnikow holding that the true function of asparagine is to hold and bind toxic ammonia.

Thomas (1927) has made an elaborate study of the nitrogenous metabolism of apple trees. The distribution of nitrogen in the water-soluble fractions of both leaves and shoots was investigated at intervals throughout the growing season, and in a subsequent season a comparison was made between fertilised and unfertilised trees, *i.e.*, those receiving a heavy dressing of sodium nitrate and others not treated in this way. The changes throughout the year are interesting. When growth is rapid the nitrogen tends to migrate from the leaves to the shoots, where it is stored in the phloem. During bud formation the reserve protein is transported to the actively growing centres as amino-acids. In the autumn there is migration of nitrogen from the leaves to the branches and it is stored mainly in the first and second year shoots. The evacuation of nitrogen from leaves in the autumn has always been a matter of controversy, and is seemingly now settled by this work.

**Nitrogen Relationships of the Ripening Seed.** Elaborated nitrogen enters the developing seed from the centres of synthesis and is resynthesised into reserve protein. According to Woodman and Engeldow (1924), who have studied the development of the wheat grain with special reference to the synthesis of proteins in the grain itself, no trace of nitrate could be discovered during the formation of the grain, and they concluded that nitrogen must enter the grain as soluble organic nitrogen, which is then resynthesised to protein *in situ*.

### The Nitrogen Metabolism of the Leguminosæ

The position of the Leguminosæ is peculiar with regard to their nitrogen metabolism. It has been proved beyond all doubt that these plants can assimilate the free nitrogen of the atmosphere. This is due to the presence of the bacterium, *Bacillus radicola*, in the nodules on the roots of these plants. This organism has been shown by Bewley and Hutchinson (1920) to pass through a very definite life-cycle. They showed that the cycle started with non-motile cocci which swelled and developed flagella. The next

stage consisted in the loss of the flagella and the bacteria again became non-motile and vacuolated. In the final stage the bacteria showed a banded appearance and then passed into cocci once more or developed flagella (Fig. 33). This work has been confirmed by Thornton and Gangulee (1926), who showed that the same series of transformations occurred in the soil as well as in artificial culture. In any sample examined, all these cell types occur, but their proportions vary at different times, and the changing percentages in the first twenty-seven hours is shown in

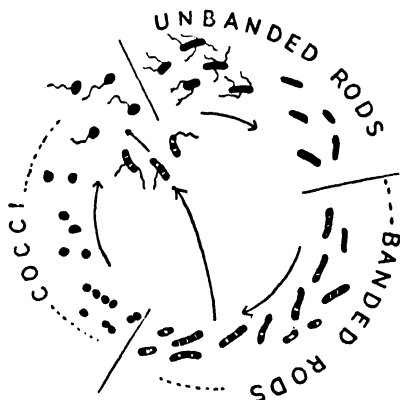


FIG. 33.—The Life-cycle of *Bacillus radicola*. (After Thornton and Gangulee, *Proc. Roy. Soc. Lond.*)

Fig. 34. Within this period the population passed through two complete cycles of change, each commencing with an increase in the proportion of cocci and banded rods. In both cycles, the maximum percentage of cocci was reached as soon as, or before, that of the banded rods from which they arose. The presence of di-acid calcium phosphate (0.1 per cent.) in milk hastened the predominance of the cocci and increased their percentage. The presence of calcium also affected the migration of the bacteria through the soil and helped in their wider distribution. The effect was chiefly shown towards the tips of the roots rather than near the seed. The precise influence of the phosphate was not discovered.

The presence of minute amounts of boron also have an important effect. In the absence of boron the bacterial nodules do not develop extensively and the bacteria tend to assume a parasitic habit (see Chapter IV).

The bacteria enter the plant *viâ* the root-hair near the tip and multiply with considerable rapidity, and this is followed by much

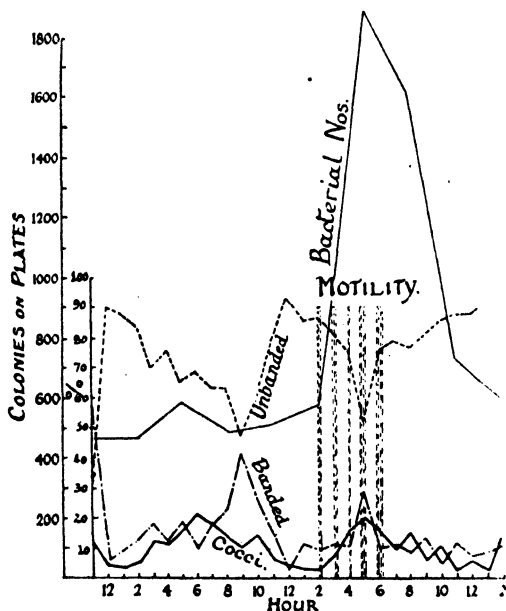


FIG. 84.—The total number of organisms and percentages of cocci, banded and unbanded rods, at two-hourly intervals in culture. (After Thornton and Gangulee, *Proc. Roy. Soc. Lond.*)

softening of the wall of the hair. They are strictly confined to the inner cells of the cortex. Strands grow down from the main vascular system and connection is made with the circulating system of the plant. During entry the bacteria pass through the cycle described above. The non-motile cocci enter the root-hair as swimmers. Once in the interior of the hair, they become rods, and finally become branched banded rods in the nodule.

Thornton (1929) has found in *Medicago sativa* that the first

appearance of nodules coincides with the expansion of the first true leaf. To determine whether these two events are casually connected, seedlings of *M. sativa* were grown in test-tubes containing plant food solution inoculated with a suspension of the nodule bacteria. In one series, the leaves were cut off as soon as they appeared; in the second series, the cotyledons were cut off but the true leaves allowed to develop; and in the third, control series, the true leaves and cotyledons were left. On the control plants the appearance of the nodules followed the opening of the true leaves. In the series with the cotyledons cut, the opening of the true leaves followed a course similar to that shown for the control plants, but the development of nodules was delayed until considerable growth of true leaves had taken place. This indicates that the passage into the root of substances from the cotyledons is one of the factors controlling the early appearance of nodules. But in the cases where the true leaves were removed, the normal course of nodule appearance was not significantly altered. The substance affecting nodule formation is therefore not produced in the true leaf at the time of its expansion, since its action is unaffected by the removal of the leaf. It would seem, therefore, that the opening of this leaf coincides with some other change in the physiology of the seedling, resulting in the extrusion of the substance which stimulates infection by the nodule organism.

It was also found by Thornton (1930) that if the host legume were grown in darkness so that photosynthesis cannot take place and the normal supply of carbohydrates to the nodules is cut off, the bacteria become parasitic and obtain their necessary energy requirement by consuming the host nucleus, cytoplasm and even cell wall. Lack of carbohydrates, however, will not entirely account for this parasitic attack. The question arises here, Why do not the bacteria consume both carbohydrates and host cell contents normally? There must be some other factor present which limits the bacterial population of the nodules to a size which can obtain its required energy from the normal supply of carbohydrates from the leaves. Air supply may be one such factor. Seedlings grown in agar, for example, were weak in growth; the bacterial population was small, but did not assume the parasitic

habit as the supply of carbohydrate was sufficient for the limited size of the population. It is also a well-known fact that bacteria in older nodules become parasitic, and this parasitism shows obvious resemblances to the active parasitism brought about by shortage of carbohydrate. It is possible that this result is brought about by lessened photosynthesis with advancing season.

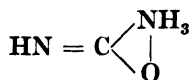
The chemistry of nitrogen fixation is still quite unknown. The plant is supposed to be supplied with soluble protein, but the stages whereby the molecular nitrogen of the atmosphere is transformed into complex protein have still to be solved.

### The Function of Urea in the Plant

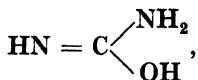
It is a well-known fact that urea plays an important part in the metabolism of animals. It is chiefly in the form of this compound that the nitrogenous waste products of protein metabolism of animals are removed in the urine. The presence and function of urea in plants, however, has always been a matter of controversy. The enzyme, urease, however, which eventually decomposes urea into ammonia and carbon dioxide, has a wide distribution in the vegetable kingdom, especially among the Leguminosæ, and if this enzyme be present, it would appear improbable that urea is not present as well.

Urea is undoubtedly present in the fungi. Iwanoff (1923) has made a number of quantitative investigations on this question and shown that urea is present in *Lycoperdon saccatum*, *L. piriforme*, *L. gemmatum*, *Bovista nigrescens*, and *Psalliota campestris*. For example, as much as 11 to 16 per cent. of the dry-weight of *B. nigrescens* was found to be composed of urea. The urea was shown to be absent in the early stages of development and increased to a maximum with age. In *Lycoperdon*, Iwanoff showed that the urea was formed from available ammonia, and considered that its function in the fungi was to act as a nitrogen reserve. Fosse (1916, 1920) claimed that urea is also present in the higher green plants, and demonstrated its presence by the delicate xanthidrol reaction.

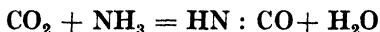
E. A. Werner (1922), who has succeeded in showing that urea possesses the cyclic constitution :—



which can in certain circumstances give the open ring structure :—

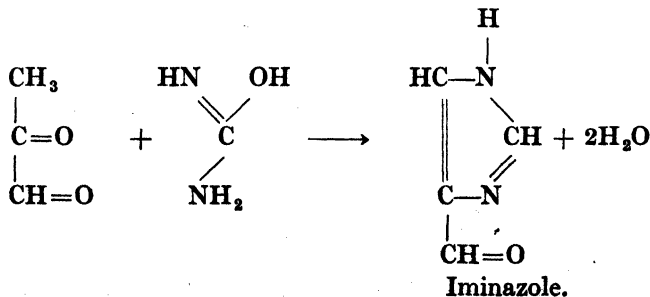


considered that in the germinating seedling when the assimilation apparatus has become fully established cyanic acid is built up from carbon dioxide and ammonia according to the following equation :—



and that the subsequent condensation of this cyanic acid with nascent carbohydrates is the first step in the synthesis of proteins. It has been known for a long time that plants can assimilate urea from culture solutions, but not the substituted ureas, such as ethylurea. It is possible that plants cannot utilise the substituted ureas on account of the fact that they do not possess any enzyme in their cells capable of hydrolysing these bodies to ethylamine and cyanic acid, whilst the superior action of urea over ammonia in this respect is due to its power of immediately supplying cyanic acid.

It is possible that urea may play a part in the synthesis of purine derivatives, compounds with heterocyclic rings in their molecules. For example, the iminazole ring could be synthesised in the following way from one molecule of urea and one molecule of methylglyoxal by elimination of two molecules of water.



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## CHAPTER IV

### THE RAW MATERIALS OF PLANT NUTRITION

*The Elements Needed to Build up Plant Tissues—Carbon—Nitrogen—Phosphorus—Potassium—Calcium—Secondary Elements in Plant Nutrition—Iron—Boron—Manganese—Silicon.*

#### **The Elements Needed to Build up Plant Tissues**

THE green plant is a synthetic machine, elaborating its own food material from relatively simple inorganic compounds which it obtains from the atmosphere and the soil. These initial substances are often loosely referred to as the actual food materials of the plant. This, however, is not the case, they are the raw materials from which complex food material is constructed through the metabolic activities of the plant.

Plant tissues are composed principally of the elements, carbon, hydrogen, oxygen, nitrogen, as well as sulphur, phosphorus, calcium magnesium and potassium. Besides these principal components, it is becoming increasingly apparent that particular elements are necessary in minute amounts to obtain normal growth in certain plants, and these appear to subserve a similar purpose as the so-called vitamins in the animal economy. The qualitative study of the mineral nutrients necessary for plant growth is usually carried out in water culture solutions by leaving out one of the necessary elements. The method has the inherent weakness that the physiological balance of the solution is disturbed and the question of antagonism enters into the problem.

The effect of deficient nutrient media on the photosynthetic activity of the plant has been investigated by G. E. Briggs (1922). Using *Phaseolus vulgaris*, whose seed contains a large initial store of food and therefore is very suitable for such an investigation, Briggs grew plants in full nutrient media and also in media lacking the

elements, potassium, phosphorus, iron and magnesium, respectively. In each of the cases tested, in which one element of the normal solution was absent, the assimilatory activity of the leaves was discovered to be markedly less than the leaves of plants grown in a complete solution. This subnormal photosynthetic activity was found to be present in each case to the same extent when either light or temperature was playing the part of a limiting factor.

### Carbon

The only source of carbon for the higher green plants is the carbon dioxide of the air. This is present in the atmosphere in minute amount, approximately 3 parts in 10,000, but this low concentration is sufficient for the synthesis of carbohydrate. It has usually been considered that material increase of the carbon dioxide concentration has a toxic action on plants. This result, however, is due to the fact that the older workers employed impure sources of the gas, such as those from blast furnaces and gas flues. The carbon dioxide from such sources is always mixed with considerable amounts of sulphur dioxide and hydrogen sulphide, which are markedly toxic to plants. Bolas and Henderson (1928), using highly purified carbon dioxide and working under rigidly controlled conditions, found that artificial enrichment of the atmosphere with carbon dioxide resulted in a large increase in the dry-weight of cucumber as compared with the controls in normal air. The increase became evident at an early stage of the growth, usually two to three days from the beginning of the experiment. In one experiment, with a concentration of 81.3 parts of carbon dioxide per 10,000, the plants showed a percentage increase in dry-weight of  $60.6 \pm 8.5$  over the controls with 3.9 parts of the gas in 10,000. These results will doubtless be of value in greenhouse horticulture in the future, and further observations should be of interest.

Certain of the lower green plants, especially the green algæ of the soil, can function as either autotrophic or heterotrophic organisms in nutrition. Bristol Roach (1926, 1927, 1928) found that the soil alga, *Scenedesmus costulatus* var. *chlorelloides*, could be

grown in a liquid medium of nutrient mineral salts alone in the light or with the addition of 1 per cent. of different carbohydrates in the dark. With the addition of 1 per cent. glucose the organism was able to grow in the dark and retained its green colour. With maltose a similar result was obtained, but there appeared to be an initial "lag" period, preceding the straight line period of growth; xylose, however, was completely toxic to the organism. In the light, glucose and maltose gave the best growth, while the order of other carbohydrates was: galactose, sucrose and fructose. Other algæ which were found to be heterotrophic were: *Cystococcus* (sp.), *Chlorella* (sp.) and *Chlorococcum* (sp.), but they reacted very differently to the conditions imposed upon them. It is probable that it is not justifiable to regard the soil algæ as a homogeneous physiological unit in consideration of their relation to soil fertility. With *Scenedesmus costulatus* var. *chlorelloides* it was discovered that in high light intensity the organism was completely autotrophic in nutrition, but as the light intensity was reduced, glucose was taken up from the medium. It is possible that when this alga occurs in the lower layers of the soil, it grows at the expense of certain organic substances that are directly available. When, however, growing on the surface layers of soil, provided that the moisture conditions are suitable, photosynthesis takes place at a rate dependent on the intensity of the light.

### Nitrogen

The main source of nitrogen for the green plant is nitrates. Nitrogenous manures promote growth, their action is very swift, and they make the leaves a vivid green colour, causing rapid growth of leaf and stem. In large quantities they lead to rank and heavy growth and, in wheat, a tendency to susceptibility to infection from rust. Hursch has found that the amount of sclerenchyma is reduced in proportion to the collenchyma in wheat with heavy nitrogen manuring, thus favouring the attack of *Puccinia graminis*, the mycelium of which can only develop in the collenchyma. Gregory (1926) has shown that barley increases its leaf area but not its assimilation rate with addition of nitrate,

whereas addition of phosphorus and potassium increase both assimilation rate and leaf area.

. Appleton and Helms (1925) have ascertained that the rate of absorption of sodium nitrate by oats and cotton when applied at different stages of growth is more rapid the later the nitrate is applied. In both cases there is a close correlation between the rate of growth and the rate of nitrogen uptake. The effect of the amount of nitrogen supplied has been studied by Rippel and Ludwig (1926) on the growth of the sunflower; for smaller rates of nitrogen supply the actual rate of growth is less, but in the first half of the growth curve the relative production of dry matter and the relative absorption of nitrogen are greater with the smaller dressings. Rippel and Ludwig (1925) have also attempted to correlate uptake of nitrogen with that of bases in the broad bean and oats grown in sand culture with or without the addition of combined nitrogen. The excess of nitrogen in the plants named above, which could have been absorbed in the form of nitrates combined with the bases present in the tissues (allowance being made for bases combined as sulphates and phosphates), is expressed as a percentage excess of nitrogen. As would be expected, this figure is much higher for broad beans when combined nitrogen is withheld. In the case of oats, the excess is still greater when nitrogen is given, whereas nitrogen-starved oats show a large excess of bases. Turner (1926) has demonstrated the contrast between the response of barley and maize compared with flax to variations in nitrogen supply. The former two crops show a large increase in the shoot to root ratio with increasing dressings of nitrate, which is due to a stimulation of the growth of the tops, and not to a depression of root formation. In the case of flax this effect is not very marked. Beyond a certain low level of nitrogenous dressing, further increases in nitrogen supply produce no appreciable change in the ratio of tops to roots.

Pearsall and Ewing (1929) have shown that plants grown in a medium containing large amounts of nitrates possess little protein, while low amounts of nitrogen lead to high protein-content. It is obvious, therefore, that the succulent condition of the tissues of plants grown under rich conditions of nitrogen

manuring cannot be due to imbibition of water by protein. On the other hand, the ratio of protein to soluble nitrogen is always lower in plants receiving abundant nitrogen, *i.e.*, they have larger amounts of soluble nitrogen. The amino- and amido-nitrogen is always higher in high nitrogen manuring, and the chlorophyll content is also higher. The *pH* of the plant tissues under conditions of high nitrogen manuring is higher than with scanty nitrogen supply. This perhaps may be correlated with the fact that such plants as sorrel manufacture relatively larger quantities of organic acid, *e.g.*, oxalic acid, in soil deficient in nitrogen. The water-content of the tissues with high nitrogen is always considerably greater than with low nitrogen. Pearsall and Ewing suggested that since abundant nitrogen leads to an accumulation of amino-acids, these influence the metabolism in such a way that the production of organic acid is reduced, leading to an increase in the *pH* of the tissues (*i.e.*, alkalinity). This high *pH* and high amino-acid content leads to a greater swelling of the protoplasmic colloids, and it is perhaps due to this feature that there is higher water-content and reduced transpiration of plants growing under conditions of high nitrogen manuring.

### Phosphorus

The best source of phosphorus for plants is in the form of phosphates. Phosphates promote root growth and have an important effect on the ripening of grain. The function of the phosphorus in the cell is difficult to ascertain. The presence of phosphorus appears necessary for mitotic division, possibly on account of the fact that phosphorus forms an integral part of the nucleus.

It is a well-known fact that the soil factors governing the supply of phosphate to the growing plant are considerably different from those concerned in the supply of most other nutrients. Von Wrangell (1926) distinguished three separate factors for the supply of phosphate from the soil : (1) the phosphate concentration of the soil solution ; (2) the rate at which this concentration is restored after disturbance of the equilibrium between soil and soil solution ; (3) the total reserve of available phosphate in the

soil. The first factor is considered to be of especial importance, because here one is dealing with easily available material as well as with substances of low solubility, the concentration of which largely depends on the presence or absence of other ions. Mac-Gillivray (1927) has found that in phosphorus-starved tomatoes there is a re-utilisation of the phosphorus present ; about half of the total amount is found in the fruit, irrespective of treatment, although, if there be a shortage of phosphorus, the size and number of fruits is much decreased.

Brenchley (1929) has investigated the influence of phosphate on barley at different periods of growth. The plants were grown in water culture with full phosphate allowance and also with no phosphate. The plants grown in full phosphate solution were deprived of phosphate after varying initial periods and *vice versa*, *i.e.*, plants grown in the absence of phosphate were supplied with the salt after initial periods of deprivation. Provision of phosphate for the first six weeks allowed normal growth to take place, shown by the number of ears, tillers and grain produced, as well as dry-weight. Shorter initial periods of phosphate allowance led to serious shortage in these respects. If the phosphate were withheld for four weeks, tiller growth was not affected, but no ears were produced. With still longer initial periods of deprivation, growth was steadily depressed in all respects, and the usual bushy growth tended to pass over to the thin, lanky type. The amount of phosphate absorbed by the plant increased more or less in direct proportion to the length of time phosphate was given at the beginning of growth, but sufficient was taken up in the first six weeks to allow of the plant making maximum dry-weight. The absence of phosphate in the early stages of growth, on the other hand, led to an extremely rapid drop in the ultimate amount of phosphate taken up by the plant.

It is evident that the importance of phosphate lies in the early stages of growth, and it is then that its application is of the most vital importance. Provided a sufficiency of phosphate be supplied in the early weeks of growth, tillering, ear, grain formation and increase in dry-weight proceed normally. These results are in contradiction to those of Pember (1917) and Pember and

McLean (1917), who found that barley can remedy a deficiency of phosphate at any time in the life of the plant. This, as Brenchley points out, may well be due to the fact that they used well water in their experiments, which contains traces of phosphate, and, further, that they did not allow the tillers but only the main shoot to develop, so that such phosphate as was present was concentrated in its sphere of action.

Gericke (1925), working with wheat, obtained the maximum dry-weight when the plants were grown in nutrient solutions for four weeks and then transferred to solutions containing no phosphate. Decrease occurred in the ultimate dry-weight as the initial period with phosphate was lengthened; the plants receiving phosphate throughout the experimental period being amongst those with the lowest dry-weight. Brenchley never found this sharp fall in barley, although certainly there was decrease in the dry-weight with continuous supply of phosphate.

It would seem that the phosphate requirements of cereals at different times of development are by no means fixed or definite but are influenced by various factors, such as environmental conditions (seasons, spacing of plants, time of sowing, etc.). Nevertheless, it seems to be a definitely established fact that the most critical period for phosphate nutrition is in the early stages of growth.

### Potassium

Potassium aids in the production and translocation of carbohydrate and appears to be of especial importance to leguminous plants. Penston (1931), who has investigated the distribution of potassium in the potato plant by means of microchemical tests, claims that, within the cell, potassium is localised in the cytoplasm and vacuoles. Potassium was never discovered to be present in the nucleus. With the exception of dead cork cells, potassium was found to be present in all cells, the greatest concentration of the element being apparently in the apical meristems of shoot and root, the outer region of the cortex, especially in the stem, and it was also present in the leaves, phloem and reproductive organs.

Maskell (1927), in a series of observations on the production of starch in the potato, using a special technique which allowed observations to be made in the field, found that in plants growing on plots, which had received respectively potassium chloride, potassium sulphate, low-grade potash salts and no potassium, statistical examination of the data showed that starch production was appreciably increased by potassium sulphate, but not by fertilisers containing potassium chloride. The rate of translocation of starch from the leaflets on the potassium sulphate plots was also increased, but at the same time this varied significantly with other factors, of which, intensity of solar radiation and age were important. Further evidence that carbohydrate metabolism and potassium content are related was found by Janssen and R. P. Bartholomew (1929, 1930), who showed that plants are able to absorb more potassium than is actually used in their metabolic processes. In the various plants investigated, *e.g.*, soybean, oat, cotton, cowpea, etc., it was shown that there is a close relationship between the percentage of potassium and the percentage of carbohydrates.

According to Gregory and F. J. Richards (1929), barley (variety, Plumage Archer) grown in sand culture with full manuring, nitrogen deficient, phosphate deficient, or potassium deficient, gave the following results :—

<i>Respiration.</i>		<i>Assimilation.</i>	
		(Low light intensity.)	(High light intensity.)
Fully manured.	Normal.	Unaffected by age of plant.	Falling with age.
N <sub>2</sub> deficient.	Subnormal.	Normal. Unaffected by age.	Subnormal. Falling with age.
PO <sub>4</sub> deficient.	Normal.	Slightly supernormal and falling with age.	Slightly supernormal. Falling with age.
K deficient.	Supernormal.	Subnormal.	Subnormal.

They further found that the absence of potassium has less effect on tillering than absence of either nitrogen or phosphorus, and this tillering proceeds till death is about to take place. Rapid death of the tillers now occurs, and after this the new leaves which appear on the surviving tillers are darker green in colour, and from this time onwards the death of the leaves keeps pace

with new development, so that at most two living leaves are found on each tiller. It was discovered that the death of the leaves sets free potassium, which is immediately translocated to freshly developing leaves.

In a continuation of this work on the effect of potassium on the assimilation and respiration of barley, F. J. Richards (1982) has now found that as the level of external potassium concentration is lowered, there is an increase in the respiratory rate. The cause of this increase in respiration rate with increasing potassium starvation is not clear. Richards suggests that under conditions of low potassium supply and ample nitrogen, a high percentage of nitrogen in the plant may be anticipated, leading possibly to a larger percentage of protoplasm in the potassium deficient leaves than in the leaves of the fully manured plants. Since other workers have reported (see for example, Nightingale, Schermerhorn and Robbins) that there is an increase in total nitrogen under conditions of potassium deficiency, it is considered in this work as a preliminary hypothesis, that the increased respiration of leaves deficient in potassium may be attributed to the increase in amino-acid content. There is a certain amount of concrete evidence available for this view. Spoehr and McGee (1923) found that amino-acids have an accelerating effect on the respiratory rate of leaves.

The relation of potassium to nitrogen and carbohydrate metabolism has been investigated by Nightingale, Schermerhorn and Robbins (1980) for the tomato. Plants grown in the absence of sufficient potassium began to show symptoms typical of the initial stages of nitrogen starvation. The stems became stiff and woody, growth was slow, no new growing points were formed in the leaf axils, and finally, the plants were much stunted. Microchemical tests revealed that accompanying these growth responses there was heavy deposition of starch in the parenchymatous cells of the cortex, phloem and medullary rays. It was also found that nitrates were abundant in all parts of the plants. In the control plants, on the other hand, the growing stems were soft and succulent, and although sugars were present in moderate amount, starch was low, and limited almost solely to the endodermis. The

anatomical structure of the stems was in striking contrast to the potassium-starved plants. There was much less xylem, which was thin walled, and the cambium was active even at the base of the stem. The pericyclic fibres, which were thick walled and conspicuous in the potassium deficient plants, were so thin walled in the stems of the plus potassium plants as to be distinguished only with difficulty from the adjacent parenchymatous cells. Comparison of the nitrate content of plus and minus potassium plants showed that the former assimilated all the nitrates in their tissue, whereas the minus potassium plants maintained a high concentration of nitrates in all parts up to the time of death. Nightingale and his co-workers consider, therefore, that potassium is essential for the synthesis of organic nitrogen from nitrates. It was found, for example, that on the application of potassium to minus potassium plants, considerable quantities of nitrites made their appearance in the phloem, as well as in the cortical tissues of stems and veins. This formation of nitrites immediately following upon the application of potassium appears to indicate that potassium is specifically necessary, either directly or indirectly, for the initial stages of nitrate assimilation.

Penston (1981) is also of the opinion that potassium is closely associated with protein formation. From a series of micro-chemical tests on the potato plant, which have already been briefly alluded to above, she ascertained that there was a close association of potassium and protein in the meristematic regions where protoplasm is being actively synthesised, and again in such tissues as outer cortex, phloem, palisade and protein storage tracts of the tuber, and she is of the opinion that this is clearly indicative that potassium is a factor in protein metabolism.

There appears to be at the present time a good deal of controversy with regard to the relationship between succulence and potassium supply. Janssen and Bartholomew (1929, 1980) found that among the variety of plants that they used for their investigations, those grown in a medium containing a large amount of potassium were more succulent than plants grown on a nutrient solution only containing a small amount of potassium. "The data presented show quite conclusively that high-potassium

plants are more succulent than low-potassium plants" (Janssen and Bartholomew). Similarly, Nightingale, Schermerhorn and Robbins (1930), in their work on tomatoes, also comment on the fact that plants grown in the absence of potassium showed decreased succulence, while Tincker and Darbishire (1933) have found that plants of *Stachys tubrifera* deprived of potassium wilt more easily than those grown in the presence of this element, although no difference could be observed in the moisture content of the tissues as a result of potassium starvation. James (1931) also claims to have found a close relationship between potassium and water content in the potato plant. Gregory and F. J. Richards (1929), and also Richards (1932), report that under their experimental conditions the leaves of barley under potassium deficiency are more succulent than in its presence. As Richards has pointed out: "The divergence between the two sets of results can scarcely be due to the differences in the plants used, . . . and the author has obtained increased succulence under reduced potassium, not only with barley, but also consistently with leaves and stems of three species of grasses." It is possible that the explanation may lie in the varying nature of the nutrient media employed by these different investigators. Further evidence for this view is shown by the fact that both Janssen and Bartholomew, as well as Nightingale and his co-workers, report that the leaves of tomato plants are darker green under potassium deficiency, whereas Gregory and Richards found that in barley the leaves are of a light yellow-green under conditions of potassium deficiency.

### Calcium

The absence of calcium causes stunting and discolouring of roots, as well as brown spotting and subsequent death of leaves. Day (1929) has studied the effect of calcium deficiency on the anatomy of *Pisum sativum* and found that the plants were stunted and the lower leaflets and stipules had become chlorotic, except at the edge and at the base of the main vein, which remained fairly green. The leaflets and stipules in the upper parts of the plants showed decided signs of curling and became rigid almost

to the condition of leathery toughness. The internal structure of the plant, however, did not appear to be affected.

The older physiologists associated calcium with the formation of proteins as well as with the formation of the material composing the cell wall. Recent work has tended to confirm this view. Parker and Truog (1920) explained the association between protein and calcium as being due to the precipitation of acids formed as by-products in protein synthesis by this element; for example, the Leguminosæ, which are rich in protein, are also rich in calcium. J. D. Newton (1923) did not support the conclusions of Parker and Truog. He found that the intake of calcium by peas and wheat in water culture solution is not proportional to the nitrogen content. Newton explained the high calcium content of leguminous plants when grown in the soil to be due to the much larger amounts of carbon dioxide evolved by the roots of such plants in comparison with non-leguminous plants, and that this results in an increased uptake of calcium which does not necessarily bear any relation to the nitrogen content of the plants. The argument, however, is not entirely conclusive, and the subject requires further examination.

The relation of calcium to assimilatory activity and nitrogen metabolism has been examined by Nightingale, Addoms, Robbins and Schermerhorn (1931) for the tomato. The plants were originally grown in loam and later shifted to washed quartz sand and watered with the requisite nutrient solutions employed in this investigation. The calcium-deficient plants presented a very characteristic appearance. The upper portions tended to a yellowish colour while the lower parts remained a fairly dark green. There was cessation of meristematic activity, followed by death of this region. The plants showed marked accumulation of carbohydrates with high concentration of soluble sugars and starch, and the latter was widely distributed throughout the plant. The most characteristic feature associated with calcium deficiency, however, was the fact that the plants were unable to absorb nitrate to any great extent, a result which may account for the relationship between calcium and protein synthesis. Nightingale and his co-workers distinguish between *combined* and *uncombined*

calcium. The latter can be detected by the usual tests for calcium, *e.g.*, oxalic acid, whereas the former must first suffer a preliminary hydrolysis with alkali before it can be detected. It is possible that the combined calcium is united with protein or in some other way. It was found that in plants grown in the dark there was decrease of carbohydrates, a result to be expected; there was also hydrolysis of protein, an increase in uncombined calcium and a decrease in combined calcium. At the same time, there was an increase in the absorption of nitrates and rapid formation of new tissue. Calcium-deficient plants, when supplied with this element, rapidly took it up, and this was followed by absorption of nitrates.

Neither barium nor strontium can replace calcium, as both these elements are extremely toxic to the well-being of the plant.

### Secondary Elements in Plant Nutrition

It has become increasingly apparent in recent years that besides the elements enumerated above as being essential in plant nutrition, other elements in minute amount are also necessary for the normal growth of the plant. The early pioneering investigations of Mazé (1916) foreshadowed the possibility that, by more refined methods, the ten elements postulated by Knop and the older school of physiologists as satisfying all the requirements of plant growth might be insufficient. This view has been abundantly justified by a number of recent investigations.

The method of experimentation is to grow plants in the usual nutrient solutions prepared from highly purified chemicals. In such circumstances many plants fail to grow normally. The addition of minute amounts of certain elements, *e.g.*, zinc, manganese, aluminium and boron, has, in a number of cases, brought about normal growth.

### Iron

Although iron does not form an integral part of the molecules of the chlorophyll pigments, nevertheless a small amount of iron is necessary for the production of these pigments in leaves.

In 1920, Oddo and Polacci put forward the claim that iron could be replaced in culture solutions by the magnesium salt of pyrrole carboxylic acid. This statement, however, could not be substantiated by Deuber (1926), who found that in no case did the substitution of the magnesium salt of this acid prevent chlorosis in cowpea, soybean, corn and *Spirodela*. In fact, in concentrations of 0.001 to 0.250 gm., the salt was definitely toxic to the plants.

### Boron

It was shown by Warington (1923) that the broad bean, *Vicia Faba*, only attained full development when grown in the presence of a trace of boron, and that no other element could replace it.



FIG. 35.—Broad beans (*Vicia Faba*) grown in the presence of various concentrations of boric acid. Left to right, 1 in 5,000; 1 in 50,000; 1 in 100,000; 1 in 500,000; control with no boric acid. (After Warington, *Ann. Bot.*)

In the absence of boron the plants showed a stunted development, and death eventually occurred in a very characteristic manner by the blackening and withering of the tips (Fig. 35). The

best results were obtained with amounts of the order of one part in a million, while larger quantities (1 in 5,000) were toxic. Warington came to the conclusion that boron is essential for other leguminous crops, but not for barley. Brenchley and Thornton (1925) have found that the nodules surrounding the roots of *Vicia Faba*, which are responsible for nitrogen assimilation, are much



FIG. 36.—Nodule of *Vicia Faba* without strands, in which bacteria are attacking the tissues. At (a) masses of bacteria have broken the cells of the host and turgid uninfected cells can be seen projecting into the disintegrating tissue. (After Brenchley and Thornton, *Proc. Roy. Soc. Lond.*)

reduced in the absence of boron. The vascular structure becomes defective, the strands being either entirely absent or only running for a short way into the nodule; there is at the same time a reduced development of bacteroid forms, and the amount of nitrogen assimilation is much below normal, and, in fact, the bacteria have been found to change their symbiotic habit for one of parasitism, attacking the protoplasm of the host (Figs. 36 and 37). Warington (1926) has confirmed this work of Brenchley and

Thornton, and found considerable hypertrophy of the cambial cells of *Vicia Faba* in the absence of boron, as well as frequent disintegration of phloem and ground tissue and poor development of xylem.

Brenchley and Warington (1927) have carried the matter a stage further, and have discovered that the need of boron is independent of the pH of the medium in which the plants are

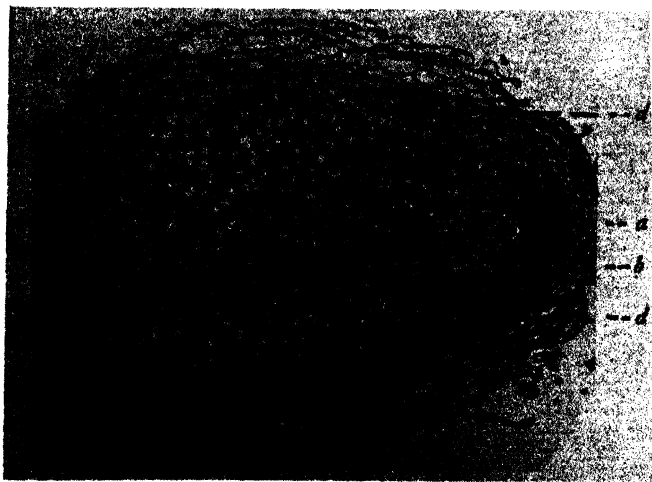


FIG. 37.—Longitudinal section of large nodule of *Vicia Faba* showing normal structure in the presence of boron ; (a) meristem cap, (b) swollen vacuolated cells, (c) bacteroidal tissue, (d) vascular strands. (After Brenchley and Thornton, *Proc. Roy. Soc. Lond.*)

growing. Even an insoluble borate such as aluminium borate is effective in this connection. Boron is essential for several leguminous plants as well as melon, whereas cereals and candytuft can develop in its absence. The boron plays an integral part in the calcium metabolism of the plant ; in its absence *Vicia Faba* is unable to assimilate calcium.

Collings (1927) stated that, contrary to Warington's results, boron is not essential for the complete development of the soybean, although in water culture it does have a markedly stimulating

effect. Sommer and Lipman (1926) found that boron is essential for the development of corn, peas, sunflower, vetch, barley, buckwheat, dahlias, lettuce, potatoes, millet, castor beans, sugar beet, kafia, sorghum, flax, mustard and pumpkin. They ascertained that dicotyledons respond more quickly to boron than do mono-

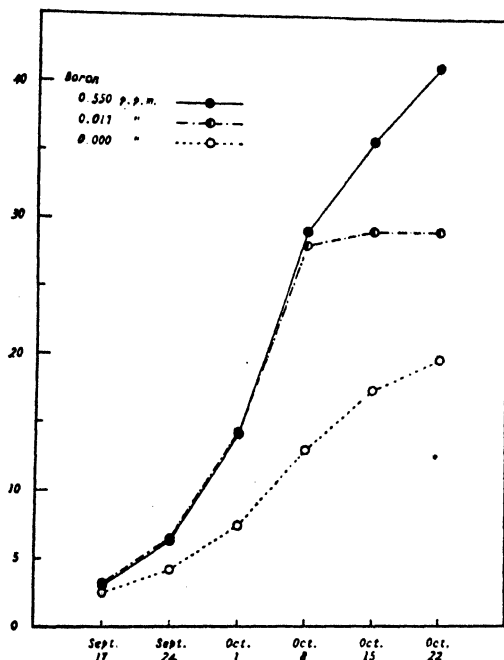


FIG. 38.—Average height (centimetres) of tomato plants grown in nutrient solutions deficient in boron and in solutions to which 0.011 and 0.55 parts per million boron had been added. (After Johnston and Dore, *Plant Physiol.*)

cotyledons, and it may well be on this account that Warington failed to find any stimulating effect of boron on barley. The effect in certain cases may not become apparent for a week or two, or even for as long as a month (barley). They also found that zinc is necessary for wheat, buckwheat, broad beans and kidney beans.

Johnston and Dore (1929) have discovered that boron is essential for the normal growth of the tomato. In the absence of this element the plants show four distinct types of injury : (a) death of the terminal growing point of the stem ; (b) breaking down of the conducting tissues in the stem ; (c) characteristic brittleness of stem and petiole ; and (d) roots extremely poor in growth and of a brownish unhealthy colour. There appears to be evidence of a quantitative relationship between the amount of growth and the amount of boron present in the nutrient medium : the greatest amount of growth was obtained when the concentration of boron was 0.550 part per million (Fig. 38). It has been further shown by Johnston and P. L. Fisher (1930) that the tomato is a plant which requires a continuous supply of boron throughout its life-cycle in order to maintain normal, healthy growth. Plants were grown in culture solutions containing 0.5 part per million, and unless boron was kept continuously supplied, stem growth fell away, the tips turned yellow and died, and the most characteristic feature of lack of boron in this plant, stem-brittleness, made its appearance, and finally the fruits became covered with blackened and dead areas. It would appear from this work that boron, in some manner at present not understood, becomes fixed in the tissues of the tomato and cannot be utilised over and over again.

### **Manganese**

Minute amounts of manganese have also an important effect on the growth of certain plants. McHargue (1926) grew maize, onion, cucumber, lettuce, wheat, spinach, oats, beans, tomato and peas, in pot culture, in quartz sand specially freed from manganese salts. Two series of experiments were set up : (1) in which plants were grown with the addition of manganese carbonate ; and (2) in the absence of any manganese compounds. In the controls, when the manganese contents of the seeds principally contained in the testas and embryo were exhausted, growth ceased, and the leaves showed signs of chlorosis, and the starch and sugar content also fell. Normal growth was

resumed when the manganese was added to the soil. Neither copper, iron, zinc nor arsenic was able to replace manganese in this respect, and McHargue considered it to be of equal importance to iron in the formation of the chlorophyll system.

Bertrand and Rosenblatt (1922) have found that leaves can be divided into four classes. In the first, which includes beet and *Aucuba*, the highest manganese content occurs in the youngest leaves. Later in the life of the plant the manganese content of the plant falls. In the second group, which includes box, ivy, iris and yew, the manganese content at first decreases and then rises to a higher value than before. In the third group, for example, *Syringa*, privet and chestnut, the manganese content increases at first very rapidly to a maximum, and this value then falls, while in the fourth and last group there is a steady increase in manganese content with age. Further investigations of Bertrand and Rosenblatt have shown that in *Nicotiana rustica* and *Lilium lancifolium*, the highest manganese content occurs in those organs which are most active biochemically. Thus, the flowers possess more manganese than the leaves and young leaves more than older leaves and green stems.

McLean (1927) claimed that manganese salts pumped into leaves *viâ* the stomata by means of a porometer are equally as effective as when they are added to the soil. Schreiner and Dawson (1927) have discovered that if tomatoes be grown in the field and also in pot culture, using a naturally calcareous soil practically free from manganese, the addition of 25.5 parts per million of manganese sulphate produces normal growth in contrast to chlorosis in the untreated soils.

There appears to be a good deal of controversy as to whether manganese is essential for the normal development of *Lemna*. N. A. Clark and Fly (1930) could not find any increase in reproduction when they grew *L. major* in concentrations up to 1 part in a million, while in higher concentrations, manganese proved to be definitely toxic. On the other hand, McHargue and Calfee (1932) definitely state that manganese is essential for normal reproduction to take place in *L. major*.

## Silicon

The older physiologists considered silicon to be of importance in the nutrition of the Gramineæ, and that its presence gave strength to the straw. Later experiments, however, have shown that silicates act by causing an increased assimilation of phosphate. Lemmermann and Wiessmann (1922) considered that perhaps silicates or colloidal silica could replace or partially replace phosphates. This view has since been shown to be untenable, and Lemmermann himself has abandoned it. Lemmermann, Wiessmann and Sammet (1925) have now published the results of a further investigation, and have shown quite definitely that the favourable effect of silica is to be correlated with an increased assimilation of phosphates. Němec (1927) has found that the amount of phosphoric acid taken up by the plant is proportional to the amount of water-soluble silicate in the soil.

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## CHAPTER V

### TRANSLOCATION

*Introduction—Path of Translocation—Dixon's Views on Translocation—Translocation of Carbohydrates—Nitrogenous Products—Mineral Salts.*

IN the higher plants the food material elaborated during active metabolism must be transferred from one region of the plant to another for purposes of storage. Thus, sugars synthesised in the leaves are removed to other parts of the plant body and stored as insoluble polysaccharide for future use. Similarly, proteins have also to be translocated away. Again, salts from the soil solution must be passed up through the plant for a variety of anabolic purposes. It follows, therefore, that paths of passage must exist in the somatic organisation of the plant, so that this process of translocation may be facilitated.

It is important to bear in mind that translocation of material is regulated by supply. Thus, substances present in sufficient quantity may undergo very rapid translocation until the needs of the plant are satisfied. Moreover, different substances may be translocated in different directions at one and the same time. In the germinating seed, the reserve stores of food in cotyledon or endosperm have to be conveyed in two different directions: (1) to the upwardly developing shoot, and (2) to the downwardly developing root.

In the case of annual plants, growth ceases towards the end of the season, and translocation of elaborated food material becomes a serious problem, inasmuch as the ripening fruit and seeds must be supplied with sufficient reserve stores for successful germination in the following season. In annual plants, then, there is an upward translocation to maturing fruit and seeds, while in perennials the reverse holds good, for the elaborated food is passed

down to the underground rhizomes, roots, bulbs, and similar organs, for storage during the winter months of the year. They are utilised from this source when growth recommences in the spring. Translocation is now in the reverse direction, and the stored food, after it is brought into a suitable state for removal by the action of hydrolysing enzymes, is carried upwards into the newly developing buds.

In the higher plants the internal tissues may be roughly divided into wood or xylem which is dead tissue, and the bast or phloem, as well as ground parenchyma, which are living cells with protoplasmic contents. The question arises, Which of these tissues carries away the elaborated food material from the active centres of synthesis?

The older investigators considered that the function of the xylem was to carry water and dissolved salts, while the phloem furnished the channel for the downward passage of the elaborated products of metabolism. The chief evidence for the phloem being the channel of transport of the already synthesised organic food material was largely based on "ringing" experiments. It has long been known that if a stem were "ringed," then the passage of food apparently ceased. The main line of translocation was considered to be through the sieve tubes; the companion-cells and phloem parenchyma merely played a subsidiary part. Thus, the products of photosynthesis and protein synthesis were primarily mobilised in the leaf, and were then removed to the storage organs *via* the sieve tubes.

The early work of Czapek appeared to give complete confirmation of this picture of translocation, and his experiments are too well known to need more than the very briefest reference here. Czapek, for example, showed, that if the petioles of leaves were killed either by steam or with chloroform, the depletion of carbohydrates was completely stayed, or, if the petioles were cut on one side, there was considerable delay in the removal of carbohydrates in that half of the leaf. On the other hand, if the petioles were immersed in a 5 per cent. solution of potassium nitrate, carbohydrate transport was not interfered with. It was concluded that plasmolysis of the phloem did not interfere with the transport

of sugars, and the sieve tubes were the chief organs of transport because the deposition of callus synchronised with the stoppage of translocation.

It was not until 1911 that any serious criticism was brought against this work. In that year Deleano stated, that if the petioles of leaves were killed even in such circumstances, carbohydrate still left the leaf, although there was considerable reduction in the rate.

H. H. Dixon and Ball (1922) seized upon the criticisms advanced by Deleano, and concluded that the upward as well as the downward transport of organic material was through the wood; a view further elaborated in 1922 by Dixon in his Presidential Address to the British Association (Section K).

Dixon, in the course of his address, pointed out the well-known fact that Fischer had found reducing sugars as well as proteins to be present in the xylem, and, further, that he (Dixon), and Atkins, had shown that the sugars in the wood were not only hexoses, but that disaccharides such as sucrose and maltose were also present, and that these carbohydrates were to be found in the tracheæ at all times of the year. Dixon considered that ringing experiments are of little value, inasmuch as the surface of the wood may be injured, and, more important still, may become blocked with air-bubbles into which substances are exuded by morbid changes in the cambium, medullary rays, and wood parenchyma.

However, the more cogent of Dixon's arguments in this connection lie in certain calculations that he has made with regard to the actual quantities of organic material involved in transport, and the velocity of flow in the channels which is necessary to effect this transport. He gives as an example a selected potato tuber of weight 210 gm. The total cross-section of the bast in the slender branch was  $0.0042 \text{ cm.}^2$ —no allowance having been made for the thickness of the cell walls. In 100 days all the material in the tuber must have passed through this cross-section. On analysis it was found that 24 per cent. of the tuber was combustible; therefore, more than 50 per cent. of carbohydrate passed through this conduit. Assuming the average concentration of carbo-

hydrate to be 10 per cent., the volume necessary to convey 50 gm. must be 500 c.c., and its velocity must have been :—

$$\frac{500}{0.0042 \times 100 \times 24}$$

i.e., nearly 50 cm. per hour. As a matter of fact, the concentration is probably never higher than 5 per cent. and possibly not more than 4 per cent. Dixon considered that the nature of the sieve tubes, with their thick, viscous contents, did not admit of such a rapid rate of flow.

Dixon further thought that there was a downward as well as an upward flow of water in the xylem and cited two experiments in support of this view. A petiole of *Sambucus nigra* was split longitudinally and one half removed, and the attached portion was then placed in a solution of eosin. It was found that the solution was rapidly drawn up the wood capillaries of the intact half petiole and soon appeared in the veins of the pinnæ on the same side of the leaf, beginning with the lowest and gradually working up into the upper ones, and finally into the terminal pinna. In the next stage the dye worked its way through the veins of the pinnæ on the other side, and eventually to the lowest pinnæ on the split side of the petiole. The second experiment of Dixon consisted in cutting the tip of an upper leaf of a potato plant under a solution of eosin. The liquid was quickly drawn back into the tracheæ of the leaf and passed down the stem even into the tuber.

Both experiments, however, are open to criticism. In the case of the leaf of *Sambucus nigra*, it would be expected under the experimental conditions employed, that the dye would be drawn up one series of pinnæ and would eventually pass down the other side. This, therefore, is no proof of the reversal of the transpiration stream. That a backward suck was exerted by the potato leaf is also not surprising if Dixon's cohesion theory is correct. One of the main points of the cohesion theory is the fact that the water is considered to be under a strong tensile strain. In such circumstances, if the strain at one point were broken in any way, the strain under which the water is suffering in the other tracheæ

would tend to cause a suck back and show apparent reversal where in reality none existed.

According to Dixon, translocation can be best explained on the cohesion theory of the ascent of sap. "Transpiration, by drawing off water from the tracheæ, causes water filling these tracheæ to pass into a state of tension. This tension determines a flow from any source wherever situated and the continuous column in one series of tracheæ to draw down solution in a neighbouring filament of tracheæ terminating above in some local supply."

Although Dixon's view that the xylem and not the phloem was the channel of transport was accepted for some time after its announcement, there nevertheless has been a growing opposition to the theory, and the most recent work on the subject shows quite definitely that it is the phloem which is the channel of transport of carbohydrates. In support of Dixon's views, Arndt (1929) has reported that there is an upward as well as a downward movement of eosin solution in the same ring of xylem in the coffee tree. It was found that the solution usually moves in the outer ring of the xylem. One-sided staining, discovered when the eosin was applied to the lateral roots, indicates that specific roots are connected with particular branches of the crown. In daylight there is a more rapid upward current of eosin, and it is only in the late evening, at night, and in the early morning, that the downward movement is not greatly reduced. Arndt considered that this downward movement is quite adequate to account for the downward transfer of solutes.

The most persistent critic of Dixon's views was Curtis, who, in a series of papers (1920, 1923, 1925), maintained that the phloem served as the channel for the transport of elaborated food material. It was shown by Curtis that in ringed stems protected with paraffin wax there was a considerable interruption of the upward supply of carbohydrates as well as nitrogen and ash to the growing regions of plants, whereas only very small amounts of xylem were necessary for the supply of water.

By the use of divided stems whereby water was supplied to the top by one set of roots and nitrogen by another, Curtis was able to show that the nitrogen was not transferred by the xylem, while

if the xylem were connected by a short strip of phloem, then a normal supply would be translocated upwards. The experiment, however, was not of a very satisfactory nature, and by a later series of experiments he was able to show that the xylem alone was not satisfactory as a channel of transport for nitrogen, while the phloem alone was quite as satisfactory as xylem and phloem together.

Dixon's calculations based on the potato tuber is certainly a difficult point to explain, but, as Curtis pointed out, no sugars have ever been found in the xylem of the potato, and, further, measurements based on the work of Artschweger indicate that the cross-section of the xylem is smaller than the cross-section of the phloem of the tuber. Curtis agreed with the view put forward by de Vries many years ago that the rotation of the protoplasm helps in the conduction of food down the phloem, and he considered that protoplasmic rotation is not a pathological phenomenon as some investigators have assumed, and cited the cases of *Nitella* and *Elodea*, where the streaming exhibited by the protoplasm is undoubtedly a normal phenomenon of the cell.

If, according to Curtis, the phloem supplies an efficient mechanism for the upward supply of carbohydrate, nitrogen, and ash, then it is probable that it is just as efficient for the downward transport of metabolic products from the leaf.

A different method was adopted by Weevers (1923), in order to determine whether the upward transfer of foods during periods of terminal shoot growth takes place through the xylem or the phloem tissues. In this investigation ringing experiments were conducted on the branches of variegated species of *Aesculus Hippocastanum* and *Acer Negundo*. The use of branches bearing leaves with no chlorophyll eliminated the possibility of the manufacture of food above a ring, which would occur in normal green leaves, thus obviating the necessity for defoliation or for darkening of this part. The ring wounds were protected with wet bandages or by the application of cocoa butter. Experiments were carried out in the spring just before the buds started growth and also in midsummer. The experimental procedure consisted of the complete ringing of green and yellow shoots and the partial ringing of yellow shoots.

In either spring or midsummer, complete ringing of the shoots prevented normal growth, while partial ringing of the yellow shoots or complete ringing of normal green shoots allowed considerable growth to take place. Analysis of *Æsculus Hippocastanum* showed 8 per cent. of reducing sugars in the green leaves, 1 per cent. in the yellow from unringed branches and only traces from the ringed branches. When ringed, the leaves from the yellow branches often showed early withering and death. Though no variegated branches were available, comparable experiments were conducted with *Nerium Oleander*, which bears phloem strands internal to the secondary xylem. Ringed branches, darkened by enclosure with blackened paper, continued to grow, indicating that ringing in this case had not prevented food movement. This work shows very clearly that removal of the phloem, contrary to Dixon's statements, does very seriously interfere with the translocation of carbohydrate.

The most conclusive work on the subject of translocation has been carried out by Mason and Maskell in Trinidad on the cotton plant. This work, which embraces both carbohydrate translocation as well as translocation of nitrogenous products and ash, is based entirely on quantitative data and probably represents the most brilliant series of plant physiological investigations during the years 1928-31. It will be necessary to consider this work in its various aspects in detail, as it is the only authoritative pronouncement at present on this particular branch of plant physiology, but the original extensive memoirs should also be consulted, as they are admirable examples of lucidity of expression combined with care in the interpretation of results.

### Carbohydrate Translocation

Mason and Maskell (1928a, 1928b) estimated the quantity of carbohydrate in the bark, leaves, and wood of the cotton plant at different times of the day. They expressed their results as a percentage of the "residual dry-weight," *i.e.*, dry-weight less total carbohydrates. The employment of residual dry-weight in this connection as a basis of calculation is, on theoretical grounds,

to be preferred to total dry-weight, since the major fluctuations in dry-weight of an organ are due to fluctuations in carbohydrates. It would therefore seem to be more reasonable to make the assumption that the remaining fraction of the dry-weight will be relatively constant over short periods of time.

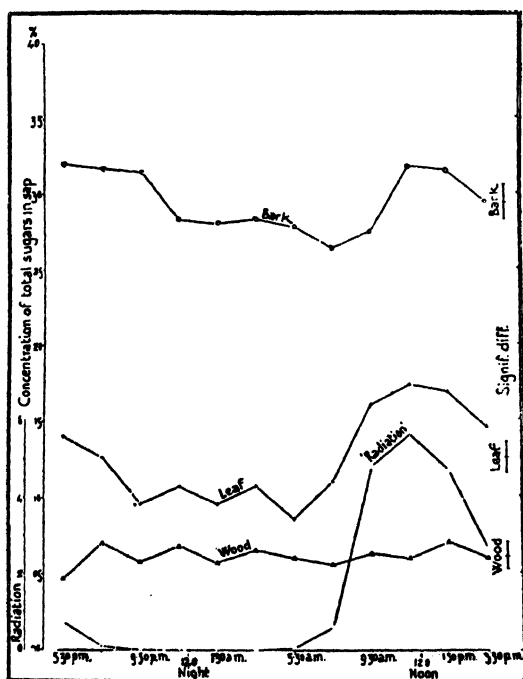


FIG. 39.—Graphs showing the concentrations of total sugars in bark, wood, and leaves of the cotton plant. Changes in the sugar content of the bark are very similar to those in the leaf, but tend to lag behind the latter. (After Mason and Maskell, *Ann. Bot.*)

Fig. 39 gives the curves for the diurnal fluctuations in total sugars together with the curve for radiation. It will be seen that while the curves for leaf total sugars and bark total sugars follow a similar course and can be correlated with the curve for radiation, total sugars in the wood show no significant variation.

The concentrations of reducing sugars and sucrose in the sap of laminæ and bark as well as wood are shown in Fig. 40. A curious

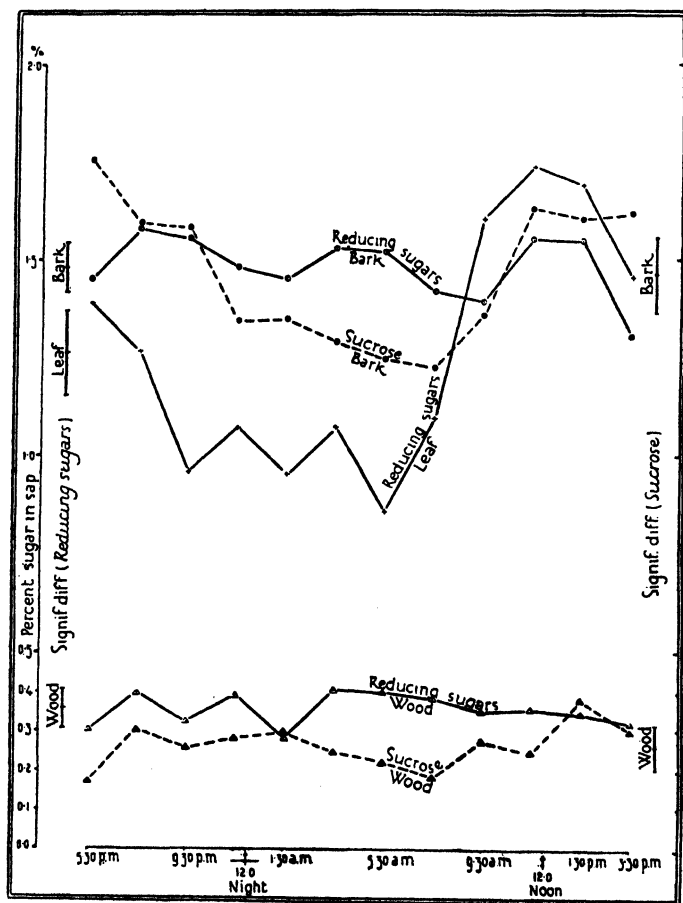


FIG. 40.—Concentration of reducing sugars in sap of leaf, bark, and wood, and of sucrose in sap of bark and wood. Grm. sugar per 100 c.c. of sap. The "significant differences" for reducing sugars are shown on the left, and those for sucrose on the right. (After Mason and Maskell, *Ann. Bot.*)

result was obtained in this set of diurnal observations; *no* sucrose was discovered in the leaf-blade throughout the experimental

period, so that the curve for reducing sugars in this instance is identical with that of total sugars. It will be seen from the curves that in the xylem the reducing sugars altered but little during the experiment. The sucrose in the wood, however, does show a significant variation. When we turn to a consideration of the bark tissues, it will be observed that the reducing sugars follow no particular pattern, but that the variation in sucrose is considerable and follows a definite course, falling at night and rising during the day. It is evident that if sucrose be the sugar of translocation, and that sugars are translocated in the bark as sucrose, changes in the concentration of this sugar in the bark might be anticipated, whereas if carbohydrates first travel downwards in the outer layers of the wood and are subsequently moved from thence to the bark, the diurnal changes discovered in the sucrose of the bark might merely indicate that sucrose is a temporary storage product.

The relationship suggested by the curves between sucrose concentrations in lamina and bark and seeming lack of relationship between lamina and wood receive confirmation from the relative magnitude of the correlation coefficients. These are shown in Table II. :—

TABLE II.—CORRELATIONS BETWEEN SUGAR CONCENTRATION IN SAP OF LEAF AND SUGAR CONCENTRATION IN SAP OF BARK AND WOOD. (After Mason and Maskell.)

Correlation between Concentration of Total Sugars in Leaf and Concentration of	1. Direct Correlation.	2. Partial Correlation, allowing for Trend with Time.	3. As 1, but Leaf Values shifted 2 hours.	4. As 2, but Leaf Values shifted 2 hours.
Total Sugars in bark	+0.4626	+0.7559	+0.7894	+0.9404
„ „ wood	+0.1907	+0.0109	+0.8831	+0.4498
		P=2.6%	P=9.1%	P=1.0%
Sucrose in bark	+0.5827	+0.7868	+0.9146	+0.9773
„ „ wood	+0.3652	+0.2044	+0.6510	+0.6723
		P=4.4%	P=6.0%	P=0.5%

(Significant correlations are shown in heavy type.)

It will be seen from column 1 that the only significant direct correlation is that between leaf total reducing sugars and sucrose

in the bark. From the curves, however, it will be noticed that the general drift in time of the sugars in all three tissues is not the same, and by calculating the partial correlations with time constant the effect of this difference between the three tissues in their general drift with time may be eliminated. It will be seen that when this is effected the leaf-bark correlations are now greater (column 2) and both are statistically significant, whereas the leaf-wood correlations have fallen. "The curve shown for the sugar concentrations in the bark sap may thus be described in terms of a diurnal fluctuation closely correlated with the diurnal fluctuation in the leaf and superimposed on a general downward drift; the drift being due presumably to a high ratio, for the time being, of loss to gain." Since the sugar concentrations in the bark appear to lag behind those in the leaf, a higher correlation should be obtained if the leaf and bark values be shifted on a period (in this case two hours). The correlation coefficients (both direct and partial) as a result of making such a shift are shown in columns 3 and 4 of Table II. In this instance the direct and partial correlations between leaf total sugars and wood sucrose become significant ( $+0.6510$  and  $+0.6723$ ), but even in these circumstances their magnitude is far below that of the leaf-bark correlation coefficients.

It is thus evident that diurnal variations in concentration of sugars in leaf sap are in this experiment closely correlated with changes in sugar concentrations in bark sap. There is also the further fact to be considered that there is an increase in the magnitude of the correlation coefficient by advancing the leaf values by two hours, indicating thereby that changes in sugar concentration in the leaf may be reproduced within a few hours in the bark at a minimum distance from the leaf.

Mason and Maskell also calculated the correlation coefficients for the weight of sugar per 100 grm. of residual dry-weight, since it was found by them that lamina, bark, and wood all contain less water during the day than during the night, and it could be argued from this that a correlation of some kind between the sugar concentrations of the leaf and of the stem might exist as a result of moisture changes quite independently of changes in the

net amount of sugar present. These further <sup>s</sup>would follow the shown in Table III. :—

All the cha-

TABLE III.—CORRELATION BETWEEN SUGAR CONTENT OF LEAF AND OF BARK AND WOOD. (After Mason and Maskell.)  
(Sugar content as grm. sugar per 100 grm. residual dry-weight.)

Correlation between Total Sugars of Leaf and	1. Direct Correlation.	2. Partial Correlation (Time Constant).	3. As 1, but Leaf Values shifted on 2 hrs.	4. As 2, but Leaf Values shifted on 2 hrs.
Total sugars of bark	+0.1862	<b>+0.3038</b> } P=6.8%	+0.5311 }	<b>+0.3211</b> } P=5.1%
"    " wood	-0.0621	-0.0471 }	+0.1337 }	+0.2783 }
Sucrose of bark	+0.4278	<b>+0.3031</b> } P=2.8%	<b>+0.3006</b> } P=8.2%	<b>+0.3045</b> } P=2.7%
"    " wood	+0.0892	-0.0955 }	+0.4034 }	+0.4468 }

(Significant correlations are shown in heavy type.)

Although the actual correlations are somewhat smaller than those for the sap concentrations, nevertheless, the results are essentially the same. The majority of the bark correlations are significant, whereas none of the wood correlations attain statistical significance.

The effect on the translocation of carbohydrates in "ringed" stems was also examined; the exposed regions being waterproofed with vaseline. It was found that the removal of a ring of bark caused a very considerable fall in the amount of sugar *below* the ring in both wood and bark within a period of little more than seven hours. This marked fall in sugar concentration below the ring suggests that ringing operates by preventing access of sugar to the region beyond the ring. On the other hand, the effect of ringing on carbohydrates in the leaf, bark, and wood *above* the ring showed at first a marked accumulation, and this increase was followed by a decrease. It is thus evident that ringing of the stem has no effect on the machinery responsible for the movement of sugar from leaf to bark above the ring.

Further experiments with ringed stems in which the flaps of bark were separated from the wood by means of paraffined paper, showed that conduction took place at practically the normal rate.

in the bark. From between bark and wood is not necessary for normal drift in time.

Diurnal estimations of the sucrose supply to the bolls showed that translocation was four times as fast by day as by night. The variations of sugar transport into the bolls could be significantly correlated with variations in the sucrose gradient from bark to boll, and it is perhaps by means of these gradients of concentration that the supply of sugars is removed from one part of the plant to another. Samples of bark taken from different levels of the same stems showed that there was a concentration gradient in the bark down the stem towards the roots which resembled the movement of diffusion in that the direction of movement was from a region of high concentration to one of low concentration. The major part of the total fluctuations of sugars in the bark were found to be due to sucrose, and it was therefore suggested that the bulk of the carbohydrate travels in this form.

The main significant difference between physical diffusion of sugar in water and the movement of sugar in the plant is that this movement is enormously greater in the latter. It is, in fact, forty thousand times as great as the diffusion constant in a 2 per cent. solution of sucrose in water, and is almost identical with the diffusion constant for molecules the size of sugar molecules diffusing in air.

Although Mason and Maskell use the term "bark" for the channel of transport, it must be understood that this term covers all the tissues outside the xylem. The main flow of the carbohydrate was discovered to be in the sieve tubes. The question therefore arises as to whether any mechanism in the sieve tubes can be visualised which would reduce the resistance by the medium to diffusion. It is tempting in the circumstances to postulate the existence in the tubes of some special organisation of capillary structure which greatly reduces any resistance to diffusion. Nevertheless, the simple fact remains that we are quite ignorant of any such structure. A probable mechanism, at least from the physical standpoint, consists in the circulation of the contents of the sieve tubes, a view, as has already been mentioned, advocated by Curtis. A chain of sieve tubes would then form a series of

vortices and exchange of solute between vortices would follow the concentration gradient between the vortices. All the characteristics of the phenomena of diffusion, except the absolute magnitude of the rates, would be shown by this system, so that it would satisfy the known facts with regard to the movement of sugars in the plant. It is evident that there are two aspects of this mechanism. Firstly there is the movement within the sieve tubes, and secondly the movement from sieve tube to sieve tube.

It is suggested by Mason and Maskell that the former might be brought about by the streaming of the contents of the sieve tubes, that is to say, the concentration within the sieve tubes is rapidly equalised by a mass movement of the contents. The difficulty is that such a movement of the contents of the sieve tubes has not yet been observed, for the streaming of the protoplasm apparently ceases in the mature tubes. It seems also that the rate of this movement, if it should occur, would have to be greatly in excess of the rate of protoplasmic streaming observed in other vegetable cells.

That translocation takes place in living cells has been shown by some further experiments by Curtis (1929), who found that when the petioles of *Phaseolus vulgaris* are chilled between 1° C. and 4° to 6° C., the removal of carbohydrate from the leaf blades is greatly retarded or even completely stops. The temperatures which cause a check in translocation are approximately the same as those causing a cessation of protoplasmic streaming, a fact giving additional support to the theory that protoplasmic streaming is intimately connected with solute passage in the phloem. Chilling also interferes with the upward passage of inorganic material, and if the petioles are enclosed in tubes containing nitrogen under slight pressure, translocation ceases. On the other hand, removal of carbohydrate from the leaves does not appear to be appreciably interfered with when the petioles are coated with wax. This may be due to the presence of a petiolar cavity which still allows of effective aeration. This work indicates that upward as well as downward translocation of solutes, organic and inorganic, is brought about by the activity of living cells.

Mason and Maskell visualise the general picture of transport of

carbohydrates in the cotton plant as follows: reducing sugars move along a concentration gradient from the chlorophyll tissues into the sieve tubes and are there synthesised to sucrose. The leaf cells are considered to be relatively impermeable to sucrose, so that there is no leakage back to any appreciable extent into the leaf parenchyma. In this manner a considerable head of sucrose is generated in the sieve tubes of the leaf. If this represents the correct interpretation of their results, sucrose should be present in higher concentration in the mid-rib than in the lamina of the leaf, and, further, the concentration of reducing sugars in the leaf lamina should be greater than in the sieve tubes of the leaf, and this was found to be the case (Table IV):—

TABLE IV.—CONCENTRATIONS IN LEAF LAMINA, LEAF MID-RIB, SIEVE TUBES OF BARK, AND WHOLE BARK. (After Mason and Maskell.)

Hour of Collection.	Group.	Leaf.		Bark.		Leaf.		Bark.	
		Lamina.	Mid-rib.	Whole.	Sieve tube.	Lamina.	Mid-rib.	Whole.	Sieve tube.
		Total Sugars.				Sucrose.			
12.30 p.m.	Normal (1)	1.886	2.602	4.201	4.826	0.118	0.782	2.035	4.001
2.35 p.m.	Normal (2)	1.957	2.873	4.493	6.445	0.199	0.902	2.229	5.253
12.30 p.m.	Ringed	2.101	3.319	5.249	9.555	0.297	1.040	2.842	7.628
	Mean	1.815	2.931	4.648	6.949	0.205	0.891	2.369	5.627
		Reducing Sugars.				Sucrose as per cent. of Total Sugars.			
12.30 p.m.	Normal (1)	1.268	1.875	2.166	0.825	8.52	28.10	48.5	83.1
2.35 p.m.	Normal (2)	1.758	1.971	2.264	1.213	10.20	31.40	49.7	81.4
12.30 p.m.	Ringed	1.804	2.278	2.407	1.927	14.10	31.40	54.2	80.0
	Mean	1.610	2.041	2.279	1.322	10.94	30.30	50.8	81.5

Barton-Wright and M'Bain (1932), who have investigated the sugar of transport in healthy potatoes and potato plants suffering from the virus disease known as leaf-roll, are not in entire agreement with this final interpretation. These investigators found that in the normal, healthy potato plant there was a correlation between sucrose in the lamina and sucrose in the petiole. In the particular cases investigated the correlation coefficients were

statistically significant and were interpreted as showing that lamina sucrose was related to petiolar sucrose and that sucrose was the sugar of transport in the healthy potato (Fig. 41). On the other hand, it was found that in leaf-roll plants *no* sucrose was present in the aerial parts of the plant once the lamina had been left at any time during the day, or for the matter of that during any time of the growing season, but sucrose was abundant in the diseased lamina, especially towards the close of the day.

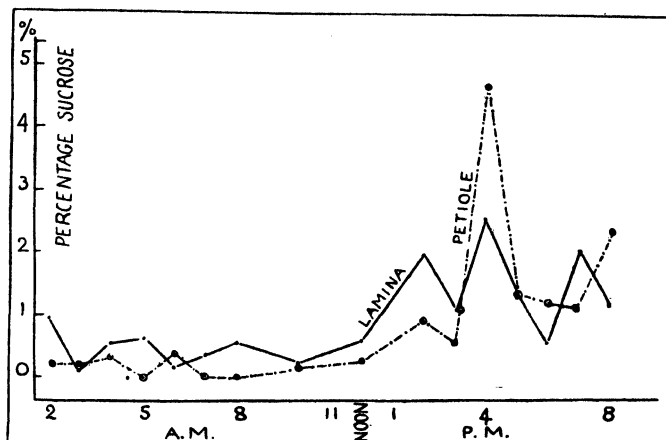


FIG. 41.—Variations in sucrose in the laminae and petioles respectively of healthy potato plants. Determinations calculated as a percentage of residual dry-weight. Potato variety used, President. (After Barton-Wright and M'Bain, *Trans. Roy. Soc. Edin.*)

In the diseased plants, therefore, sucrose cannot be the sugar of transport, and reducing sugars must perform this function. Since in leaf-roll affected plants the phloem suffers necrosis, this path of transport is blocked and only the wood or ground parenchyma of the petiole remain as possible channels of transport. It was found that reducing sugars and starch in the petiole of leaf-roll plants were directly related and could suffer conversion from one to the other, and since the starch was found to be mainly located in the ground parenchyma, it was suggested by these investigators that in diseased plants there is a slow downward movement of reducing sugars to the tubers *via* the ground parenchyma.

It will be recalled that Mason and Maskell discovered that sucrose in the lamina of the cotton plant was low in amount and often entirely absent throughout the day. As Onslow (1981) has pointed out: "... it seems doubtful whether the accuracy of the values for sucrose and hexoses is comparable with those obtained by previous investigators. . . . On the whole they do not fall into good accordance with the general trend of results. The authors (*i.e.*, Mason and Maskell) found, for instance, that the concentration of reducing sugars in the leaf is greater than that of sucrose, the latter being very low; also that the concentration of both sugars rose during the day."

The method of extraction used by Mason and Maskell was first to freeze the tissues and then to express the sap. The sap was cleared with basic lead acetate, and excess of lead was removed with sodium oxalate and the sap made up to 500 c.c. Toluene was then added as a preservative and the solution stored in an ice-chest. Such a method does not appear to be an altogether ideal procedure. Presumably it is based on the assumption that lead acetate will remove all invertase from the expressed sap. But the question at once arises, What will happen if all the invertase be not removed in this way? Hydrolysis of sucrose is bound to set in, and it may well be that it was on this account that such low values were recorded for sucrose from the lamina tissues. Mason and Maskell themselves state: "The 'frozen' sap in some cases had, relative to the total sugars, *more* sucrose than the 'alcohol' extract, in other cases considerably less." They give average values for five separate comparisons:—

TABLE V.—SUCROSE AS PERCENTAGE OF TOTAL SUGARS.  
(After Mason and Maskell.)

Tissue.	Alcohol.	Frozen.	Standard Deviation of Difference between "Alcohol" and "Frozen."
Leaf .	19.00	13.22	2.88 = 17.95 per cent. of mean sucrose.
Bark .	58.4	61.60	2.67 = 4.45     "     "     "
Wood .	71.5	71.20	1.81 = 1.84     "     "     "

It will be seen that the values given for sucrose by the freezing method are considerably less than with alcohol extraction. They also state that the sucrose values for the bark and wood are not in doubt. This is to be expected. If sucrose is the translocatory sugar, and it is travelling down the bark, it would be in the highest degree improbable that invertase would be present to any marked extent in the bark tissues.

Barton-Wright and M'Bain found in the early part of the growing season (see Chapter II) that sucrose was in excess of hexose in the lamina of healthy potato plants. Moreover, it was ascertained that the sucrose rose to very high values in the lamina of the leaf-roll plants towards the close of the day. Such a result appeared to these investigators to preclude the possibility of the sieve tubes being the seat of sucrose synthesis, more especially when the fact is considered that in leaf-roll plants the sieve tubes have suffered necrosis, and yet there is abundant sucrose in the diseased lamina.

Such criticism, however, does not invalidate the main conclusions of Mason and Maskell, and their work on carbohydrate transport will remain a classic of its kind.

### Translocation of Nitrogenous Products

Although the path of carbohydrate transport in the plant has been so successfully traced by Mason and Maskell, a large number of the problems connected with nitrogen transport are still in an uncertain state. The usual view on the subject is that nitrate from the soil is absorbed by the roots and is moved upwards in the transpiration stream through the xylem to the leaves, where it is synthesised to organic nitrogen compounds. The elaborated products are exported down the stem *viâ* the phloem. The whole problem, however, is complicated by the investigations of Curtis (see above), who claimed that not only is organic nitrogen transported by the phloem, but also mineral nitrogen.

According to Chibnall (1924), there is a diurnal variation in the nitrogen-content of the lamina of the runner-bean, the total nitrogen increasing by day and diminishing by night. It may

therefore be concluded from this work that there is a synthesis of nitrogen products in the leaf by day and export of nitrogen by night.

Maskell and Mason (1929a, 1929b, 1930a, 1930b, 1930c) have reinvestigated the whole problem of nitrogen transport in the cotton plant. In their first memoir they estimated the total nitrogen in leaf, bark and wood, and the results were expressed both as a percentage of the fresh weight (*cf.* Chibnall, 1922, 1924) and also as a percentage of the residual dry-weight (see above).

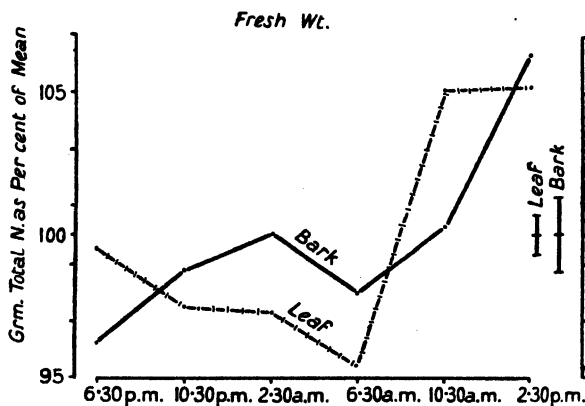


FIG. 42.—Diurnal variations in the total nitrogen-content of leaves and bark of the cotton plant. Values based on fresh weight. (After Maskell and Mason, *Ann. Bot.*)

It was found that on a fresh-weight basis diurnal variations in total nitrogen of both leaf and bark follow a definite pattern. There is a rise in the morning following upon a fall at night in the leaf. In the bark there is an accumulation of nitrogen at night and early hours of the morning, and this in turn is followed by a fall and then a further increase which is slightly later than in the leaf (Fig. 42). It was found that in the leaf the standard deviation due to sampling is markedly less than the observed standard deviation in time, so that as far as the lamina is concerned there is definite evidence of variation of nitrogen in time. In the bark, on the other hand, the observed standard

deviation in time is not much greater than that due to sampling; statistically considered therefore, there is no real evidence of variation in time in nitrogen-content of the bark tissues. But it was found that the correlation coefficient between lamina nitrogen and bark nitrogen is statistically significant ( $r = +0.6080$ ). Since lamina nitrogen and bark nitrogen are significantly correlated and lamina variation in time is in itself statistically significant, it is possible that there is a real variation in time in the nitrogen-content of the bark. Unfortunately, the small excess of the observed variation in the bark over that due to sampling makes

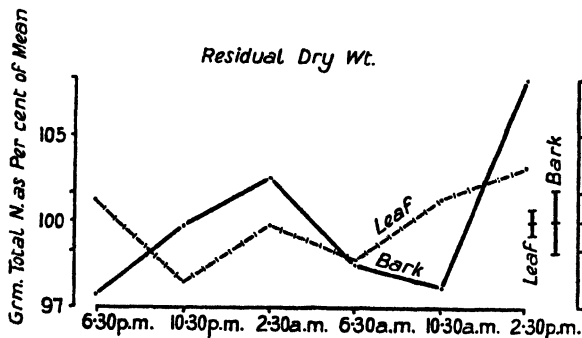


FIG. 43.—Diurnal variations in the total nitrogen-content of leaves and bark of the cotton plant. Values based on residual dry-weight. (After Maskell and Mason, *Ann. Bot.*)

this inference doubtful. There is also a suggestion from the nature of the curves that changes in the bark tend to lag behind those in the leaf, and it was found that if the leaf values are advanced one period (two hours) there is an increase in the correlation coefficient, a result in fact similar to that obtained in the case of carbohydrate translocation (see above). This increase in the value of the correlation coefficient ( $r = +0.7660$ ) by advancing the leaf values a period suggests that possibly concentration changes in the leaf lead to similar changes in the concentration of diffusible nitrogenous substances in the bark sap, and, in consequence, physical diffusion may play a part in the transport of organic nitrogen from the leaf.

The results regarded from the standpoint of residual dry-weight were by no means so satisfactory. It is true that the night figures were found to be less than the day, as was ascertained on a fresh-weight basis, but the results are less marked (Fig. 48). In the case of the lamina it was discovered that the standard deviation due to sampling is exceeded by that observed in time, but the difference is not quite statistically significant. In the bark the two standard deviations are still closer. On the whole, it can be said that while there is some evidence of variation in nitrogen-content for the lamina, whether the results are expressed on a fresh or residual dry-weight basis, this is not the case for the bark. On the residual dry-weight basis the correlation coefficient between leaf nitrogen and bark nitrogen is not found to be statistically significant, nor is the value augmented when the leaf values are advanced.

A diurnal series of estimations were also made when the plants were bolling freely. It was again found (whether a fresh or residual dry-weight basis were used) that there is an increase of nitrogen during the day and decrease during the night as far as the leaf is concerned, but no such variation could be discovered for the bark, even when the fresh-weight basis was employed. It is possible, since at this time the plants were fruiting freely, that the bulk of elaborated nitrogen was being translocated to the bolls and not to the roots.

The effects of ringing were also investigated. It was discovered that when a ring of bark was removed from the stem the leaf still continued to gain nitrogen. It may be, therefore, that the removal of a ring of bark did not interfere with the entrance of nitrate through the stem, and on this account the upward transport of nitrate from the soil through the stem is *via* the xylem and not through living tissues. It was ascertained that *above* a ring there is an accumulation of nitrogen in the bark and a well-marked diurnal variation, *i.e.*, an increase of nitrogen by day and decrease by night, whereas *below* a ring there is on the average a smaller nitrogen-content than in the untreated control plants, but the diurnal variation is not found to be statistically significant. In the case of the wood, the normal plant was not found to show

an increase of nitrogen by day or a decrease by night, such as was discovered in the bark, but in the ringed plants more nitrogen was found in the wood above a ring than below it. This would appear to suggest that there is not only a downward movement of nitrogen through the bark, but also a horizontal movement from bark to wood.

The various results obtained from the investigation of the effect of ringing by Maskell and Mason are in direct contradiction to those described by Curtis (1923), who claimed that although there was a small increase in nitrogen in stem and leaves above a ring, yet this increase was of considerably less magnitude than in the case of untreated plants. Maskell and Mason point out that this result may well be due to the prolonged period which Curtis allowed to elapse between ringing and sampling (the time period being as long as twenty-three to thirty-nine days), and that morbid changes had occurred in the wood which would lead to a serious danger of reduction in the transpiration current. They also point out, however, that in the plants used by Curtis, namely, lilac, privet and peach, the seat of nitrogen synthesis may not be the leaves, but the roots, and that the upward movement of nitrogen is a movement of organic nitrogen to the leaves. Nevertheless, until it has been satisfactorily proved that no partial blocking of the wood has taken place in this work, such a suggestion must remain unsupported.

The effect of separating bark from wood was also investigated by Maskell and Mason. It was found that nitrogen could still enter bark that had been separated from the wood, and contact between bark and wood, as was found in the case of carbohydrate translocation, is unnecessary for normal transport of organic nitrogen from leaves to roots.

A consideration of the data as a whole, presented by these authors in this first memoir, appears to be in accord with the original view of nitrogen transport, that is to say, that the bulk of the mineral nitrogen absorbed by the roots is transported up the stem in the transpiration current through the wood, while the elaborated nitrogen is removed from the centres of synthesis *via* the phloem.

Turning now to the question of the nature of the fraction or fractions concerned in nitrogen transport, Maskell and Mason have made an extensive series of observations on the subject. The problem of nitrogen transport in contrast to that of carbohydrate transport is complicated by the fact that at present it is impossible to estimate individual compounds as can be done in carbohydrate carriage, and our present methods of analysis only allow of groups of nitrogen compounds being estimated. Moreover, there is the further difficulty that the total number of these groups is greater than in the case of sugars, and, lastly, a difficulty is introduced by the unknown nature of a large proportion of the crystalloid nitrogen occurring in the plant.

The fractions estimated were: ammonia N, asparagine N, amino-acid N, nitrate N, residual N, and protein N. The term "residual N" was used to embrace the crystalloid nitrogen not accounted for by the sum of the ammonia N, asparagine N, amino-acid N, and nitrate N (see Chapter III, p. 121). The protein N was obtained by deducting non-protein crystalloid nitrogen from total nitrogen.

The first important point ascertained by Maskell and Mason

TABLE VI.—VERTICAL CONCENTRATION GRADIENTS IN THE BARK. (After Maskell and Mason.)

(Mg. nitrogen or grm. sugar per 100 c.c. of Sap.)

	5.0 p.m.	5.30 a.m.	Mean.	Standard Deviation of	
				Gradient	Mean Gradient.
Total Cryst. N. .	-118.5	-102.9	-108.2	7.54	5.83
Organic Cryst. N	-97.1	-95.1	-96.1	7.48	5.29
Asparagine N .	-87.2	-94.4	-90.8	7.64	5.40
Amino-acid + } Residual N }	-9.9	-0.7	-5.3	4.80	3.04
Nitrate N .	-16.4	-9.2	-12.8	3.09	2.18
Ammonia N .	—	+1.4	+0.7	—	—
Total Sugars .	+1.707	+1.286	+1.497	0.257	0.182
Sucrose .	+0.954	+0.851	+0.903	0.179	0.127
Reducing Sugars	+0.753	+0.435	+0.594	0.111	0.078

was in the examination of the vertical gradients in the bark. The plants were divided into an upper and lower region and the vertical concentration gradients of the nitrogen fractions are given in Table VI. ; a plus sign represents that the concentration of that particular fraction is greater in the upper region than in the lower, whereas a negative sign represents that the reverse is the case.

It is clear from the table that the sugar gradients are positive, whereas the gradients for all the nitrogen fractions with the possible exception of ammonia N are negative. Further, the major part of the gradient in crystalloid nitrogen is due to asparagine nitrogen. If there be movement of organic nitrogen from leaf to root, presumably the mobile form of nitrogen will be crystalloid in nature. A number of investigators have claimed that asparagine is the mobile form of nitrogen (*e.g.*, Chibnall, 1922, 1924). In point of fact, from Table VI. asparagine was found to form the major part of the crystalloid nitrogen fraction in the bark. But while the sugars formed a well-marked positive gradient, organic crystalloid nitrogen showed a pronounced negative gradient.

In the leaf and wood, on the other hand, it was found that there is a positive gradient of organic crystalloid nitrogen. In the leaf a positive gradient of protein is found, a feature that was not discovered in either bark or wood. Although in the main the positive gradient of crystalloid nitrogen in the wood was shown to be composed of nitrate nitrogen (rather more than half), nevertheless, there is also a positive gradient of organic crystalloid nitrogen ; a fact which strengthens the suggestion that the leaves are in dynamic equilibrium with wood and bark. In such circumstances the leaves would supply the bark with organic nitrogen, and the leaves in their turn would receive nitrate nitrogen from the wood, as well as receiving organic nitrogen from the bark according to circumstances.

When the bark was sub-divided into an inner and outer region, a marked negative gradient of amino-acid nitrogen and asparagine nitrogen was found in the inner portion, and although a negative gradient of amino-acid nitrogen and asparagine nitrogen was

also discovered in the outer region, this was by no means so pronounced.

Determinations of protein nitrogen showed that in the leaf parenchyma, leaf mid-rib, and petiole there was a great increase of protein nitrogen from the direction of the petiole to the mid-rib and from the mid-rib to the leaf parenchyma. The total crystalloid nitrogen exhibited much the same concentration in all three regions, whereas the organic crystalloid nitrogen displayed a sharp fall from leaf parenchyma to mid-rib and from mid-rib to petiole. Thus the gradient out of the leaf appears to be almost entirely due to residual nitrogen. It should also be mentioned that the gradient of amino-acid nitrogen shows almost the same gradient out of the leaf as residual nitrogen, but is smaller. The concentrations of asparagine were found to be much the same in leaf parenchyma and mid-rib, but were somewhat greater in the petiole. Finally, it was ascertained that the gradient of nitrate nitrogen was very steep from petiole to mid-rib and from the latter to the parenchyma of the leaf.

In the case of carbohydrate translocation, it will be recalled that these authors pictured a head of reducing sugars in the leaf which move along a concentration gradient from leaf parenchyma to sieve tubes, and that in the sieve tubes they are synthesised into sucrose, and that it is as sucrose that carbohydrate is translocated down the plant. In the case of organic nitrogen transport, it would appear that the data put forward support the suggestion that it is residual nitrogen which forms the necessary head for transport in the leaf parenchyma. The increase in asparagine from leaf parenchyma through mid-rib to petiole and the still further increase in the bark of the stem would thus be a phenomenon of storage. On this account, it would seem that translocation of organic nitrogen down the bark is taking place by the superposition of a dynamic over a static gradient of organic nitrogen.

The problem of transport was therefore approached from the standpoint of the relation between change in movement and change in gradient. Since the gradients of storage material may mask the gradients of translocatory movement, if transport

be stopped or its direction reversed, the dynamic gradient of translocatory material should be mainly affected. Thus, although the net gradient might still remain negative, the change in the net gradient, when the direction or rate of transport is altered, should be positively correlated with such a change in movement and should serve as a measure of the change in the dynamic gradient.

The procedure adopted to study the problem in this way was as follows : transport was brought to a standstill by ringing the stem at the base and removing the leaves, and the approach to the static condition in the leafless plants was then followed by discovering the gradients after intervals of five and eight days had been allowed to elapse. A group of plants ("leaves-on" group) ringed at the base of the main axis, but still bearing leaves in the upper part of the axis, acted as a control to the behaviour of the leafless group ("leaves-off" group). Both sets of plants were further sub-divided into an upper and lower region.

It was found that in the "leaves-off" group the total nitrogen and the residual dry-weight showed very little change in bark and wood, whereas total carbohydrates decreased considerably and the total dry-weight also fell. In the "leaves-on" group a considerable amount of growth was found to have occurred and a large mass of total carbohydrates had accumulated, while the residual dry-weight was also found to have increased. The total nitrogen also showed a marked increase, although the relative increase was found to be less than the relative increase in the total dry-weight, the difference being most clear in the upper region.

The increase in dry-weight and total nitrogen found in the "leaves-on" group, especially in the lower region, indicated that there was downward movement of carbohydrates and nitrogen, for this region bore no leaves. In the "leaves-off" group there was no appreciable movement of nitrogen.

Examination of the concentrations in the upper and lower region of the bark in the two sets of plants showed that sugar concentration increased markedly in the lower region of the "leaves-on" group, and approached the concentration found in the upper region, since synthesis of sugar in the leaves was still

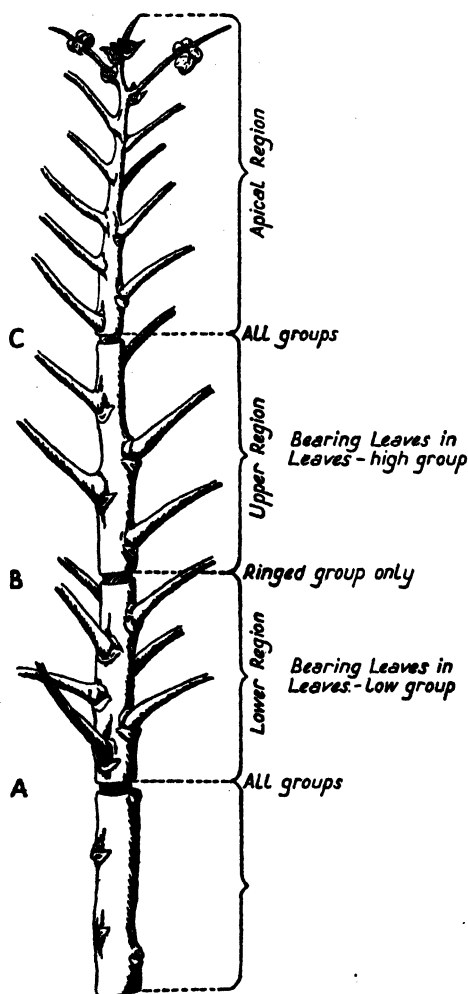


FIG. 44.—Diagrammatic representation illustrating experiment of reversing normal direction of translocation in the cotton plant. (After Maskell and Mason, *Anns. Bot.*)

taking place, and this positive gradient was still maintained to the end. The "leaves-off" group, on the other hand, showed a fall of sugar concentration in both regions, the fall being more

accentuated in the upper region until the concentration in this part reached the same low level as in the lower region. The results obtained in this experiment indicated that at static equilibrium, *i.e.*, when translocation has been brought to a standstill, the concentration of sugar was approximately the same at different vertical levels in the bark. The nitrogen, however, behaved in a different way to sugar. The vertical gradients exhibited very little change, and in the "leaves-off" group at the end of the experimental period there was still a definite negative gradient left both in total crystalloid nitrogen as well as in protein nitrogen. Thus zero movement of nitrogen was associated with a negative gradient, while zero movement of sugar was associated with zero gradient.

The gradient conditions associated with the reversal of the normal direction of translocation were next studied. In this experiment in one group of plants the upper region was made to supply carbohydrates and nitrogen to the lower region, while in the other group the lower region was made to supply the upper part. The apical region of the plant extended from the apical bud downwards to the 11th node, below this came the upper region, which consisted of five nodes, and this in turn was followed by the lower region which was composed of seven nodes. The portion of the stem below the lower region was bared of leaves (Fig. 44). Three groups of plants were used. On the day of the experiment a  $\frac{1}{4}$  inch of bark was removed immediately above the upper region and immediately below the lower region in all three groups (C and A, Fig. 44). The upper and lower regions of stem were in this way isolated from the remainder of the plant. In the "leaves-high" group the leaves were removed from the axis and branches of the lower region. In the "leaves-low" group they were similarly removed from the upper region. In the ringed group all leaves were removed from both regions, and an additional ring of bark was removed between the upper and lower regions (B, Fig. 44). In the "leaves-high" group the upper region of stem bearing leaves will supply sugars and organic nitrogen to the lower region, whereas in the "leaves-low" group the reverse will hold good and the lower region will supply the upper. The lower region of stem

in the ringed group served as a base line for the measurement of downward translocation into the lower region of the "leaves-high" group, and similarly the upper region of stem in the "leaves-low" group served as a base line for the estimation of upward translocation into the upper region of this group.

It was found that reversal of the normal direction of movement was accompanied by a reversal in the gradient of total sugars and a steepening of the originally negative gradient of nitrogen compounds. This result was interpreted as showing a change in the nitrogen gradients as the reversal of an originally positive dynamic gradient superimposed on a relatively constant static gradient. Moreover, the fact that it was ascertained that these changes took place principally in the inner half of the bark, suggests that the dynamic gradient is localised in the sieve tubes while the static gradient is principally a storage phenomenon in rays and cortex.

A further difficulty that was encountered in this work was an interconversion of crystalloid nitrogen and protein nitrogen in the bark. For example, desiccation caused a conversion of crystalloid nitrogen to protein nitrogen, whereas a decrease in sugar concentration brought about a conversion of protein nitrogen to crystalloid nitrogen.

With regard to the actual nitrogen fractions involved in longitudinal transport within the sieve tubes, Maskell and Mason considered that all fractions may take part in movement on account of the fact that the nitrogen fractions in the bark showed such marked lability, but presumably movement into and out of the sieve tubes will be confined to crystalloid nitrogen. The view put forward with regard to nitrogen transport by these investigators is as follows: as in their carbohydrate work a head in the leaf is assumed, and the residual nitrogen fraction is considered to serve this function for nitrogen transport. Within the sieve tubes all the labile forms of nitrogen, including soluble protein, take part in longitudinal movement, and the part played by each depends on the effective concentration gradient in force. The particular mechanism responsible for accelerating diffusion in the sieve tubes (protoplasmic streaming?) should in the circumstances act impartially on all material free to move.

Confirmation that residual nitrogen probably forms the necessary head was found by Barton-Wright and M'Bain (1933) in their investigation of the nitrogen metabolism of normal and leaf-roll potatoes. In the leaf-roll laminæ marked accumulation of residual nitrogen was found towards the close of the growing season. It was also ascertained that residual nitrogen probably plays an important part in longitudinal transport, since, in certain cases in diseased petioles, residual nitrogen was found to be absent, and the total crystalloid nitrogen fraction could be accounted for by the sum of the ammonia N, amide N, amino-acid N, and nitrate N (see Chapter III).

### Translocation of Mineral Salts

The question of the particular upward path along which mineral nutrients travel in the higher green plants has in the past usually been considered to be *viâ* the wood in the transpiration current. The traditional view, however, was impugned by Curtis (see above), who claimed that ash also travelled in the phloem. This claim has not been substantiated by later workers, who have been able to confirm the older interpretation.

For example, it was shown by Clements (1931) that when a number of plants such as *Vitis vinifera*, *Rubus occidentalis*, *Pyrus Malus*, and *Prunus Persica* were ringed, the upward movement of ash constituents still continued.

If the upward movement of ash be *viâ* the xylem, the question arises, Does the main bulk of the material travel with the water current to the leaf or is it abstracted by surrounding tissues during its upward journey? A further problem that arises in this connection is, If the bulk of the salts be conveyed to the leaves, what is their ultimate fate? Are they merely concentrated in the cells of the leaf parenchyma, or are they chemically altered, or are they re-exported to other parts of the plants? Finally, if re-exportation does take place, is the new channel the xylem or the phloem?

Mason and Maskell (1931) have made a preliminary survey of the transport of potassium, calcium, and phosphorus, in the cotton plant.

As in their previous work on carbohydrate and nitrogen, transport ringing of stems and also reversal of the normal downward movement was employed. With regard to the interpretation of the results of the transport of mineral substances, three possibilities have to be borne in mind : (a) If accumulation takes place below and decrease above a ring, upward movement *viâ* the phloem will be in progress ; (b) absence of any appreciable difference either above or below a ring between normal and ringed stems will indicate that under the experimental conditions no appreciable movement is taking place in the bark (phloem), but that the substances are moving upward *viâ* the wood, and are spreading from thence radially to all tissues ; and, lastly, (c) if accumulation is found above a ring and decrease below a ring, this will suggest that mineral salts, such as nitrogen, are moving upward in the wood, passing to the leaves, and then being re-exported once more *viâ* the bark.

It was found that there was no accumulation of either potassium, calcium, or phosphorus below a ring on the contrary all three decreased. Above a ring there was accumulation of potassium and phosphorus but not calcium, which fell both above and below a ring. Hence there can be no doubt that upward transport of these three mineral nutrients is *viâ* the wood. By using the reversal methods described for their work on nitrogen transport (see above), these investigators ascertained that the downward movement of potassium and phosphorus could be reversed. It would thus appear that there is upward transport of these three mineral nutrients in the xylem and downward transport of two, potassium and phosphorus, *viâ* the phloem.

When the growing bolls were removed a pronounced increase in the concentration of phosphorus and total ash was discovered in both leaves and stem tissues. Calcium was also found to show a small increase as well as carbohydrates and nitrogen. It was considered by Mason and Maskell that phosphorus, potassium and total ash travel to the developing boll through the phloem, but that calcium moves to this part in the xylem, since fertilisation was found to increase markedly the rate of uptake of phosphorus and total ash as well as carbohydrates and nitrogen by the ovule,

whereas it had little effect on calcium. Moreover, there is a similar result in the carpels, but here the difference between calcium uptake and uptake of phosphorus, potassium, and total ash, is even greater than in the ovule.

The immobility of calcium in the leaf is a curious feature of this work. Its immobility is apparently due to the fact that it is unable to move in the phloem. It was found by Mason and Maskell that calcium oxalate was abundant in the ray cells of the phloem, although tests for its presence in the sieve tubes were inconclusive. Calcium, unlike either phosphorus or potassium, is required throughout the growing period of the plant, whereas, in the case of potassium and phosphorus, these are only needed in the early stages of growth and their curtailment at an early stage does not bring growth to a standstill. Mason and Maskell suggested that calcium may gain access *viâ* the transpiration stream to all cells, but that once within the cell it is either precipitated or combined with tissue material in such a way that very few calcium ions are left in solution. A point that appears to have been overlooked is that calcium appears to play an important part in protein metabolism (see section on Calcium, Chapter IV), and it may be on this account that it is not transported out of the cells of the leaf, which, in the cotton plant, is the centre of protein synthesis.

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## CHAPTER VI

### RESPIRATION

*Chemistry of the Production of Organic Acids in Respiration—Nature of Aerobic Respiration—Factors affecting Respiration—Anæsthetics—Anaerobic Respiration and Fermentation—Oxidation Mechanism of the Cell—Glutathione—Enzymes concerned in Respiration—Oxidation of Fats and Proteins in the Plant.*

THE main advances in the study of plant respiration have been extended in a variety of different directions.

Although in normal respiration carbon dioxide and water are the end products of the reaction, it has been known for a number of years that certain succulents, notably the Cactaceæ, Crassulaceæ and Mesembryanthemaceæ, produce organic acids as the final products of respiration. This result may well be due to the massive construction of these plants, making gaseous diffusion a difficult process. The production of organic acids in respiration, however, has been mainly investigated from the biochemical standpoint in bacteria and fungi.

A curious point has been discovered in this connection, namely, that the bacteria produce monobasic acids, whereas the fungi give polybasic acids of the type of fumaric, citric and oxalic acids. This aspect of the problem has been much investigated in the ascomycete, *Aspergillus niger*. It has been known for a considerable time that this fungus produces large amounts of oxalic acid as a product of respiration. The work of Wehmer, conducted in the 'nineties, showed that not only was oxalic acid produced from glucose, but also from the salts of organic acids, such as tartaric, citric and malic acids. He found that the production of oxalic acid was independent of the supply of oxygen, but was intimately connected with temperature; the higher the temperature, the greater the production of acid. If the acid were

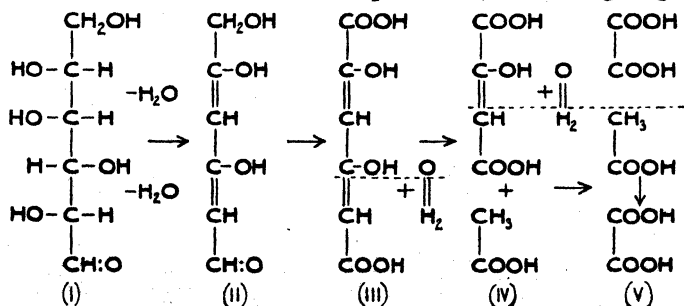
fixed in the form of the calcium or sodium salt by the addition of the carbonates of these metals to the medium, he discovered that the fungus did not appreciably attack the salt of the acid, whereas the free acid was eventually oxidised to carbon dioxide and water.

According to Currie (1917), the stages in the decomposition of carbohydrate to oxalic acid by *Aspergillus niger* are as follows :—  
Carbohydrate → Citric acid → Oxalic acid → Carbon dioxide  
→ Mycelium

Both citric acid and oxalic acid were found as products of respiration, and if the fungus were supplied with citric acid, oxalic acid was produced, so that in all probability the order of decomposition is that given above.

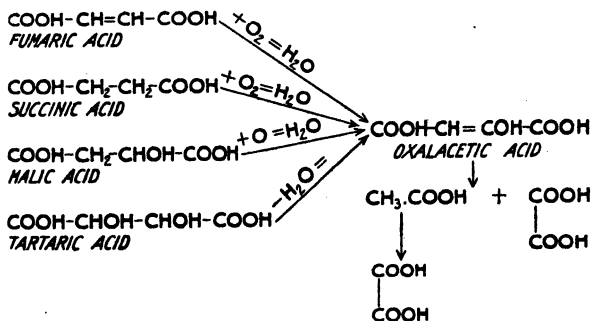
A. Raistrick and B. Clark (1919) have made a very full investigation of the formation of oxalic acid from organic acids by *A. niger*. The best growth of the fungus was found to occur when it was supplied with three-carbon acids, such as lactic and pyruvic acid, but no oxalic acid was produced. With four-carbon dibasic acids, such as succinic, fumaric, malic and tartaric acid, good yields of oxalic acid were obtained. Again, with the two-carbon acids, glycollic, glyoxylic and acetic acid, only acetic produced oxalic acid, although good growth of the mycelium was found in all three cases. When the medium contained either ammonium butyrate or ammonium propionate no growth occurred. In all the cases investigated, the four-carbon polybasic acids gave the greatest yield of oxalic acid.

Raistrick and Clark from these results argued that the breakdown of hexose to oxalic acid takes place in the following stages :—

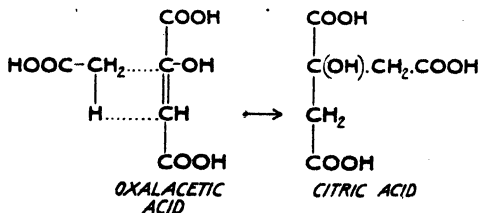


The compound (II), formed by simple dehydration, is the enolic form of a polyketide, a substance possessing the  $\text{CH}_2=\text{CO}-$  grouping in the molecule. The fact that free acetic acid was never found in the various media is the main drawback to the theory. But it must be remembered that *A. niger* has a very swift action on acetic acid and rapidly converts it into oxalic acid. This may account for its absence in the culture media.

The scheme suggested for the breakdown of dibasic acids to oxalic acid, is by the intermediate production of oxalacetic acid (IV) in the scheme given above:—

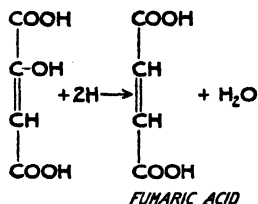


The fact that in three cases direct oxidation with oxygen is postulated and in the fourth dehydration is not a help to the theory. The production of citric acid by *A. niger* is said to be due to the addition of one molecule of acetic acid with a molecule of oxalacetic acid:—



It may conveniently be mentioned at this stage that Wehmer (1918) has isolated a strain of *Aspergillus niger* to which he has given the specific name of *A. fumaricus* owing to its ability of forming fumaric acid from hexose. Raistrick and Clark con-

sidered that a molecule of oxalacetic acid in this case undergoes direct reduction by hydrogen instead of further oxidation as in *A. niger* :—



More recently Challenger and his associates (1927) have carried out an extensive investigation of the production of oxalic acid by *A. niger*, and have evolved a complete scheme to account for the oxidation of hexose to oxalic acid. It was found that when the fungus was grown on citric and other organic acids, glyoxylic acid was produced. When it was grown on citric acid both acetone and malonic acid were discovered to be present. When cultivated on a medium with calcium acetate, glycollic, glyoxylic and oxalic acids were formed. Lastly, if *A. niger* were grown on potassium saccharate, citric acid was isolated. From these results the suggestion was put forward that the series of chemical reactions involved proceed on the following lines :—

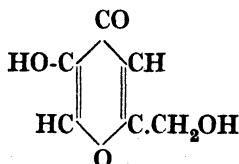
Glucose → gluconic acid → saccharic acid → β-γ-diketo-adipic acid → acetone-dicarboxylic acid → malonic acid → acetic acid (2 mols.) → glycollic acid → glyoxylic acid → oxalic acid.

Raistrick and his co-workers (1931) have now published an enormous volume of work on the products of the activity of different fungi. This investigation embraces a great variety of different groups, e.g., *Aspergilli*, *Penicillia*, *Fusaria*, etc., and as the published work extends to 366 pages, it will be impossible to do anything more here than to give a few of the salient features.

The first group to be investigated was the *Aspergilli*. It was found that the products formed by the metabolic activities of these forms and the yields obtained are only true if they receive a given amount of air. In the presence of an unrestricted air supply the

products obtained are often quite different in nature and quantity. Carbon balance sheets were prepared and in these the total carbon originally present and the amount at the end of each experiment was examined. The results obtained are arranged in the original memoir according to the classification of the *Aspergilli* given by Thom and Church (1926). With few exceptions the biochemical characteristics of any particular group as deduced from the carbon balance sheets, place the different species of *Aspergillus* in the groups assigned to them by Thom and Church. This will be made clearer by the discussion of actual examples. In the case of the *Aspergillus glaucus* group, which includes *A. glaucus*, *A. ferrugineus*, *A. medius*, *A. mollis*, *A. scheelei*, *A. disjunctus*, *A. novus* and *A. repens*, it was discovered that these various species are a closely related group from their biochemical as well as their taxonomic characteristics. Thus, they all gave poor growth on Czapek-Dox medium. Their metabolism solutions were dark-coloured, showing a marked fluorescence. They produced some optically active compound which was indicated by the fact that the glucose-content as determined by a polarimeter was considerably higher than that given by copper-reduction methods. Lastly, they all showed a uniformly high percentage of carbon in their mycelium.

A great variety of compounds are formed by moulds, depending on the species and variety. For example, *A. parasiticus*, which belongs to the *Aspergillus flavus-oryzae* group, and also includes *A. effusus*, *A. tamarii*, *A. oryzae* and *A. flavus*, kojic acid is formed from glucose. Kojic acid or 5-hydroxy-2-hydroxymethyl- $\gamma$ -pyrone possesses the following constitution :



Kojic Acid (5-hydroxy-2-hydroxymethyl- $\gamma$ -pyrone).

The formation of this substance from carbohydrates is of considerable interest from the fact that the  $\gamma$ -pyrone nucleus is a

common constituent of many groups of naturally occurring compounds. Therefore, in addition to glucose, other bodies, such as xylose, arabinose, sucrose, glycerol, mannitol, starch, galactose and fructose, were added to the medium. Kojic acid was produced from all these bodies by one or other of the species belonging to this group. The sources of carbon supply enumerated above include pentoses, hexoses, disaccharides, a polysaccharide (starch), a trihydric alcohol (glycerol) and a hexahydric alcohol (mannitol). Hence the idea originally held that kojic acid ( $C_6H_6O_4$ ) arises from glucose by simple abstraction of water, and subsequent oxidation affords no explanation of the formation of the acid from compounds containing less than six carbon atoms (*e.g.*, glycerol, xylose, and arabinose). It is possible that acetaldehyde is first formed and that this is later, by a series of reactions, condensed to give kojic acid.

### The Nature of Aerobic Respiration

According to F. F. Blackman (see Kidd, 1916), there are always two types of respiration proceeding simultaneously in the living cell. The first consists of an oxidation of carbohydrate or fat to carbon dioxide and water, to which he has given the name "floating respiration," and to the second he has applied the term "protoplasmic respiration." The latter process is considered to supply the necessary minimum of energy for the maintenance of the activity of the cell. Moreover, there are two stages in the process, the first consists in the anaerobic splitting off of carbon dioxide and some easily oxidisable substance, and the second stage lies in the oxidation of this second substance by the oxygen of the air. Thus, fundamentally considered, aerobic and anaerobic respiration have the same origin, and it is only in the end stages of the process that differences arise.

This original conception has been much extended by F. F. Blackman (1928) and his co-workers at the Cambridge Low Temperature Station by their study of the respiration of stored apples.

Blackman and Parija (1928) found that when apples are stored

they show a steady drift in respiration, which, to distinguish it from the phases of adolescence and maturity, is termed the senescent phase. In this senescent phase a fundamental change is found in the organisation of the tissues, which is considered to be due to the lowering of the normal "organisation-resistance," so that hydrolysis proceeds at a faster rate than in the phase of maturity. According to Blackman and Parija, protoplasm normally exercises a control over the hydrolysis of the respiratory substrate, and this protoplasmic control of the rate of hydrolysis is termed by them "organisation-resistance."

This change leads to a greater production of the effective substrate for respiration and so to an increased production of carbon dioxide. When this senescent phase has completed itself, respiration falls in the direction of zero by the natural starvation condition that is present in an isolated plant organ.

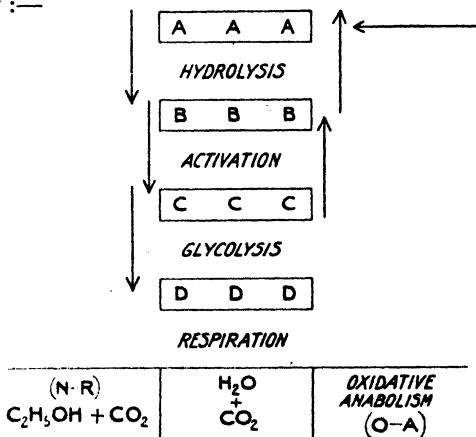
By the study of a large number of cases it was discovered that the observed respiration of an apple was due to the integration of two independent and opposed processes that were at work during senescence. One of these was a starvation drift which continuously tended to lower respiration, while the other tended to accelerate respiration by lowering the organisation-resistance, with the result that there was a rise in the rate of hydrolysis of the respiratory substrate.

Parija (1928), by a continuation of these studies in respiration, discovered that prolonged exposures to nitrogen had no disturbing effect upon metabolism. When the apples were returned to air the respiration recovered and returned to the same line of starvation drift along which it was travelling before the nitrogen was applied. The common feature, however, in all cases where the apples were transferred to an atmosphere of nitrogen, was a sharp rise in the rate of respiration. It is probable that the difference between the air condition is something other than the difference between the carbon dioxide produced by oxidation of sugar and the carbon dioxide produced by the splitting of sugar.

A survey of the respiration in nitrogen and the "air-line" respiration (*i.e.*, the respiration line in air continued to meet the respiration graph when the apples were returned to air from

nitrogen) showed that the metabolic factors which determined the progress with time were different in the two cases. It was ascertained that there was a close correlation of the initial magnitude of the nitrogen-respiration with the air-line respiration, but after that the nitrogen-respiration values followed a course of their own. One clear point emerged from these investigations, namely, that the grade of starvation in air determined whether the nitrogen-respiration values lay above the air-line respiration for hundreds of hours or for only a few tens of hours.

Blackman (1928) pictured the whole drift of the metabolites involved in respiration as a drift in a system of catalysed reactions. The scheme in this system takes the form of a chain of reactions, so that the products formed by one link become the reactants of the next link. At the free end the chain becomes branched, and we find alternate fates for the reactants controlled by the supply of oxygen. A diagrammatic representation of the scheme is given below :—



(A) includes all substances in apples which may function as reserves of carbohydrates and give rise by hydrolysis to free normal hexoses (B). Blackman assumed that the hexoses are not respired directly, but that a further carbohydrate stage intervenes, to which he gave the term "activation," leading to the formation of heterohexoses with the less stable type of internal

ring structure. This group (C) is considered as the direct substrate of the next reaction entitled "glycolysis." Glycolysis is similar in nature to the primary stage of the action of zymase in yeast which converts hexoses into ethyl alcohol and carbon dioxide, as well as a number of other substances, such as acetaldehyde, lactic and pyruvic acid, etc. The products of this reaction (D) are considered to be the reactants for the last stage, and here alternatives are possible, depending on the presence or absence of oxygen.

In the presence of nitrogen, group (D) proceeds quantitatively to the final products, carbon dioxide and ethyl alcohol, and these escape from the system as waste products. On the other hand the system behaves differently in air as well as in other concentrations of oxygen. In the first place it must be remembered that oxygen has no action on zymase activity. In air, the final products of respiration are carbon dioxide and water. Again, it has been seen that the amount of carbon dioxide produced in oxygen-respiration is less than in nitrogen-respiration; in fact, it was found that the total loss of carbon was three or four times as great in nitrogen as in air. Now, no final products of (D) accumulate in the tissues during oxygen-respiration; it is therefore logical to assume that in air part of the group is somehow worked back into the system continuously in the presence of oxygen. There is then a call for a third reactive mechanism dealing with (D), which Blackman calls "oxidative anabolism" (O—A). This anabolic building back is specific in the presence of oxygen, but short up-grade reactions occur in these linked reactions which are held to be directly reversible. Thus (B) may be converted back to (A) by condensation, or (C) to (B) by reversion. On the other hand, the stage (C) to (D) is held to be irreversible.

Further observations on the respiration of stored apples have been made by Kidd and West (1930). The course of respiration was ascertained at three different temperatures, 2.5° C., 10° C., and 22.5° C., respectively, and the carbon dioxide production followed. Progress of senescence at these three temperatures was characterised in each case by a rise, subsequently followed by a fall in

respiratory activity. The "peak" value of respiratory activity at all three temperatures was the same and about 1.5 times the initial value. It was found that the time taken to reach the "peak" value varied inversely with temperature in a particularly striking manner. This initial increase in carbon dioxide output is attributed by these authors to the action of some protoplasmic factor, and they are not in agreement with Blackman and Parija that the rise in respiratory activity is due to a lowering of "hydrolysis resistance" and an increase in concentration of respiratory substrate. Gustafson (1929) has also found a similar situation in the tomato. During growth a decrease in carbon dioxide production was discovered, and this continued until the fruit ceased from increasing in size. Following upon this period, and when the fruit was entering the senescent phase, there was an initial rise in carbon dioxide output and this then fell away. Lowering of the hydrogen-ion concentration of the sap is put forward by Gustafson to account for this result.

A peculiar effect of low temperature on the respiration of potato tubers has been described by Barker (1933). This phenomenon is quite separate from the normal lowering of respiration by low temperatures. This special depressant effect is termed by Barker "low temperature depression," and is an enduring depression of the respiratory mechanism through exposure to low temperature. The factors causing this depression are the outcome of two contrasting phases, the first of which is termed by this author "accumulation by cold" and the second "development by warmth." During the first phase Barker visualises the steady accumulation day by day of some inhibiting body at low temperatures. In the course of time this process of accumulation comes to its final limit. The lower the temperature the greater the amount of accumulation. Thus, at  $-1^{\circ}\text{C}$ . it is very large, moderate at  $+1^{\circ}\text{C}$ ., and is practically nil at  $8^{\circ}\text{C}$ . For the actual depression of the respiration rate, a second condition is needed—the development of the repressive effect of this inhibitor. This reaction is strongly affected by temperature, but the temperature relation is in the opposite direction to the process of accumulation. At  $-1^{\circ}\text{C}$ . it is very small, great at  $+1^{\circ}\text{C}$ . and very rapid at  $10^{\circ}\text{C}$ .

### Some Factors affecting Respiration

Like other physiological processes, respiration is markedly affected by internal and external factors. The chief factors which influence the rate and intensity of respiration are tabulated below :—

1. The amount of respirable material.
2. The amount of oxygen present in the air.
3. Water.
4. The amount of carbon dioxide in the air.
5. Acidity.
6. Salts.
7. Temperature.
8. Light.

The effect of a considerable number of these factors is too well known to be considered here, but important advances have been made with others in recent years, and the more relevant of these investigations will now be discussed.

The respiration of *Helianthus annuus* under field conditions has been exhaustively studied by Kidd, West and Briggs (1921). The respiration of a representative plant of a crop was determined at frequent intervals at a constant temperature. From these results it was possible to determine the respiration of a mean plant of a crop at the recorded fluctuating temperatures of the field, and thus a measure was obtained of the rate of loss of dry-weight of the plant. The effect of age could also be found from these values. These investigators were able to show that a group of internal factors is also concerned in respiration which has a marked influence upon the process.

The following factors were found to be important in their effect on the rate of respiration per unit dry-weight of the plant : (i.) the concentration of respirable material ; (ii.) the effective amount of respirable cell-matter per unit dry-weight ; the so-called " internal " factor for respiration ; (iii.) the concentration of oxygen ; and (iv.) the temperature.

This internal factor could be most readily determined when all

the other factors concerned were not limiting respiration. The respiration expressed as per gram weight of dry matter per hour when measured with respiration matter in excess, with the external concentration of oxygen equal to that of the air and the temperature at 10° C., was termed the "respiratory index." It was found that there was a continual falling off in respiration as measured by this index with the advance of age; a similar falling away was discovered in the stem, leaves and flowers. The respiratory index of the stem apex also decreased with age, indicating that the respiratory index of the meristematic tissues decreased with age. This fall in the respiratory index of the meristematic tissues and leaves indicates very clearly that the total fall in respiration in the plant with age is not due to an increase in mechanical tissue such as xylem and sclerenchyma. A close connection was found between the "internal" factor for respiration and the "internal" factor for growth.

**Water.** Since water is always present in large quantities in the cells of living organisms, it is probable that it plays a large and important part in influencing the respiratory intensity. Such has been found to be the case. Bailey (1921) found that the moisture-content in large measure determines the respiratory rate of sound corn stored under uniform conditions of temperature. With increase of moisture an increase in the respiration rate was induced; with an increase of 15 to 17 per cent. in the moisture content the respiration rate was increased by nearly 400 per cent. The respiration of corn during ripening was found to be much lower than later in the season, possibly due to a reduction in the rate of oxygen diffusion into the respiring cells and a reduction in the rate of diffusion of carbon dioxide passing out, or possibly, to a combination of both these processes. Cracked and broken corn showed an original respiration rate considerably higher than sound corn.

According to Jacquot and Meyer (1925), seeds of the broad bean, maize and pea-nut must absorb a certain amount of water before carbon dioxide is evolved. As the amount of water is increased in quantity, so there is a rise in the rate of respiration to a maximum, and this is then followed by a fall.

Meyer and Plantepol (1925) measured the oxygen and carbon dioxide exchange of mosses in the dark. The water-content influenced the intensity and variety of the oxidation. The respiratory coefficient varied with the degree of imbibition of water. When imbibition was feeble, the rate became higher than unity. The carbon dioxide evolved was the result of respiration and was not evolved from any gas dissolved in the water employed in these experiments. In dry moss plants placed *in vacuo*, as much carbon dioxide was evolved as in air. It is evident that the carbon dioxide evolved in such circumstances must have been a product of anaerobic respiration.

Richards (1927) has investigated the connection between the rate of respiration and the water-content in the higher fungi (basidiomycetes). He was able to show that with increase of water-content there was a rise in the rate of respiration. In some cases an apparent optimum amount of water was found for the respiratory rate.

**Acidity.** The acidity or alkalinity of the medium may, in certain cases, influence the rate of respiration. Gustafson (1920) studied the effect of the concentration of hydrogen ions on the respiratory rate of *Penicillium chrysogenum*. Variations in the pH value between 4 and 8 produced practically no effect on the normal rate, *i.e.*, the rate at neutrality ( $pH = 7.0$ ). Increase of the pH to 8.8 caused a fall of 60 per cent. in the rate, after which it remained constant for the rest of the experiment. With decrease in the pH to 2.65 there was a gradual rise in the respiration followed by a fall to normal. When the pH was further reduced to 1.95 to 1.10 there was a preliminary rise of 20 per cent. followed by a fall below normal. The decrease in the rate found here was ascertained to be irreversible, whereas the decrease produced by raising the pH to 8.8 was only of a temporary character, and the respiration returned to normal when the medium was changed to pH 7.0. It was also found that in acid media, *i.e.*, those with a low pH, there was a considerable increase in the consumption of oxygen, and that the reverse held good in alkaline media.

In neutral solutions of glucose and hydrogen peroxide, Gustafson (1921) found that there was an increase in the production of

carbon dioxide by *Penicillium chrysogenum* on the addition of acid, but not by the addition of alkali.

**Salts.** The action of salts on respiration depends upon their nature and concentration, and the matter is further complicated by the question of antagonism. Gustafson (1920) discovered that in a 0.05 per cent. glucose medium, the respiration of *Aspergillus niger* was increased by the addition of sodium chloride in concentrations varying between 0.35 and 0.5 molar, and also by the addition of calcium chloride up to 0.5 molar. Stronger concentrations of sodium chloride (2 M.) and calcium chloride (1.25 M.) brought about a decrease in the rate, but with a mixture of the two salts in the above concentrations and in the proportion of 19 c.c. of sodium chloride and 1 c.c. of calcium chloride antagonism was shown.

Brooks (1920) has found that concentrations of magnesium chloride solution up to 0.01 M. have little effect on the respiration rate of *Bacillus subtilis*. Well-marked antagonism was shown between sodium chloride, magnesium chloride and calcium chloride. Brooks has also ascertained that low concentrations of sodium, potassium and calcium chloride have little effect on the respiratory rate of *B. subtilis*, for the rate remained constant for hours, whereas high concentrations (0.15 and 0.2 M.) of sodium and potassium chloride and calcium chloride (0.5 M.) brought about an increase in the rate. Still higher concentrations lowered the rate. The antagonism between mixtures of sodium and potassium chloride was small, but well-marked antagonism was exhibited between either sodium or potassium chloride and calcium chloride. The antagonism curve was found to show two maxima. Of the various salts described above, potassium chloride is said to have the least toxic action in high concentrations, and is also said to have the least influence on the respiratory rate.

Brooks (1921) has also shown that the rate of respiration of bacteria is not affected by the presence of lanthanum nitrate in a concentration of  $25 \times 10^{-6}$  M. Smaller concentrations than this raised the respiratory rate, while higher concentrations lowered it. At a concentration of 0.8 M. the respiration was reduced to

zero. In mixtures of sodium chloride and lanthanum nitrate the respiration was at a maximum when the ratio La : Na was 0.2 : 99.8. With calcium chloride and lanthanum nitrate the ratio for maximum respiration was as 8 : 2, but the results were not so marked as when sodium chloride was used.

According to Lyon (1924), phosphates have a marked influence on the respiration rate of *Elodea canadensis*. Plants grown in neutral phosphate solution of concentrations 0.021, 0.085 and 0.106 M. showed a depression in respiration followed by an acceleration. Even with concentrations as high as 0.17 M. an acceleration in the respiratory rate was shown. Lyons considered that the phosphate accelerates the aerobic phase of respiration, for when the apparatus was filled with hydrogen no carbon dioxide was produced. The matter, however, is complex. In wheat seedlings, the anaerobic phase is affected as well as the aerobic. In experiments conducted over a long period it was discovered that the preliminary acceleration gradually lessened and then sharply rose to a new maximum even greater than the first, and this second steep rise was followed by a rapid fall. Plasmolysis tests revealed that the cells were dead when the curve had reached the first maximum, so that evidently there was a considerable rise in respiration after death.

Cook (1926) has tried the action of the chlorides of copper and mercury as well as silver nitrate on the respiration rate of *Aspergillus niger*. The respiration was reduced in amount and the speed of the toxic action of these salts was found to be a constant power of their concentration.

**Light.** It has been known for some time that light, besides its indirect effect of starting the photosynthetic mechanism, has a direct effect on respiration. This direct effect has usually been ascribed to the ionising action of the light on air. Using a "pure line" of barley, Middleton (1927) investigated the rate of respiration in normal and ionised air. The ionising agent employed was the radio-active element polonium. The polonium was applied for an hour, and then intermitted for an hour, and then again applied for another hour. The respiration increased during the period of application. The maximum increase regis-

tered was  $29 \pm 5.62$  per cent. during the second hour. The acceleration varied with the degree of ionisation. If the ionisation were 20,000 times that of normal air, increase in the respiration occurred from both applications of the polonium. If the degree of ionisation were 100,000 times that of ordinary air, there was only a significant increase at the second application. Still higher degrees of ionisation gave barely significant values of increase. Similarly, Whimster (1927) has observed that in the presence of polonium which gave a degree of ionisation varying between  $7.28 \times 10^2$  to  $3.64 \times 10^2$  of that of normal air, the leaves of *Pelargonium zonale* gave as high a percentage increase as  $85.7 \pm 7.1$  in the respiration rate. A curious after-effect was noticed in the succeeding two hours after the removal of the polonium, when the percentage increase rose to  $28.0 \pm 7.1$  over the control period. The effect on respiration was found to be due to the ions themselves and not to the presence of ozone. According to de Boer (1930), ionised air has no influence at all upon the respiratory rate of *Phycomyces Blakesleeanus* or *Polyporus destructor*.

### Anæsthetics

A large amount of the older work on this aspect of respiration went to prove that the action of anæsthetics was to cause an increase in the intensity of the respiration and that this increase was followed by a decrease. Gustafson (1919) found that formaldehyde, ether, and acetone, caused an increase in the rate of respiration of *Aspergillus niger*, and this increase was followed by a decrease. A 0.5 per cent. solution of caffeine also brought about a similar result. Brooks (1919) found a similar state of affairs for *Bacillus subtilis*. Here there was an increase followed by a decrease in respiration in the presence of ether. If a 0.85 per cent. solution of sodium chloride were added, antagonism was shown between the ether and the chloride. In high (8.65 to 7.3 per cent.) and low (0.37 to 1.1 per cent.) concentrations of ether, the anæsthetic proved to be toxic to the bacterium, but in intermediate concentrations it acted as a stimulant. H. S. Thomas

(1919) has ascertained that ether brings about an increase followed by a decrease in the respiratory rate of wheat seedlings. Too long exposure, over thirty minutes, results in death. Irwin (1919) showed that the petals of *Salvia* in the presence of high concentra-

tions of ether showed an increased oxygen consumption and output of carbon dioxide, and at the same time there was a decrease in the acidity of the cell contents.

A. R. C. Haas (1919) studied the action of anæsthetics on the respiration of *Laminaria*. Small doses were without effect, while larger doses gave a prolonged effect, and with still larger doses there was a slight increase followed by a decrease to zero. Haas also studied the action of anæsthetics on the respiration of *Laminaria* after death. The killed tissue was treated with such substances as ether, acetone, ethyl bromide, alcohol and formaldehyde. Using the pH method of measuring respiration (see Osterhout, 1918) and electrical conductivity of the tissues to determine their death-point (the latter was arbitrarily

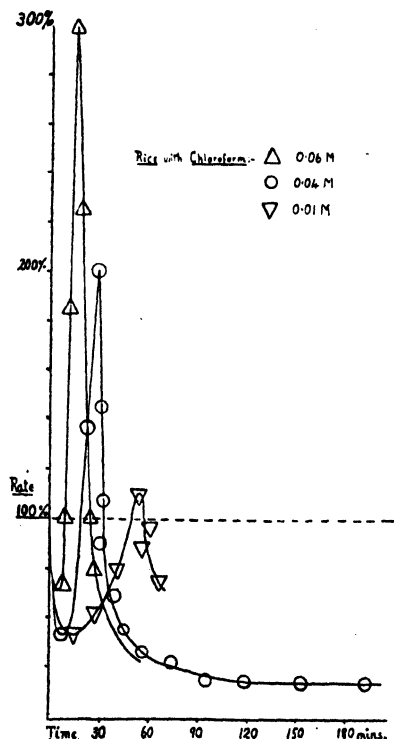


FIG. 45.—Respiration of rice with different concentrations of chloroform. (After Smith. *Ann. Bot.*)

fixed as 15 per cent. below normal conductivity), it was found that there was no particular fall in carbon dioxide output at the death-point, and it might even be higher than in the living tissues.

A fundamental contribution to the action of anæsthetics on respiration has been made by E. P. Smith (1921, 1924). Working with a "pure line" of wheat and using the pH method of measur-

ing respiration with the indicator phenol red, Smith discovered that there was an initial decrease in the rate, succeeded by a rise, and this in turn was followed by a decrease to the normal value in distilled water (Fig. 45). Smith made the claim that the action of anæsthetics is to decrease the permeability of the plasma-membrane of the cell to carbon dioxide. The carbon dioxide is held within the cell in the early stages of the process, and it is only later that it can make its way out, so that the subsequent increase shown in the curve may be partly or wholly illusory.

### Anaerobic Respiration

In the absence of free oxygen, plants are able to respire for a time, and are said to undergo anaerobic respiration. This is also sometimes spoken of as intramolecular respiration, which is not a particularly happy term. In anaerobic respiration the most frequent products of the reaction are ethyl alcohol and carbon dioxide, and the equation showing the beginning and end products may be written :—



From the point of view of the plant, anaerobic respiration is an extravagant and wasteful process, inasmuch as the ethyl alcohol produced is a waste product, incapable under the experimental conditions of undergoing further oxidation.

**Fermentation.** In its main features the fermentation of sugars by yeasts is similar to the anaerobic respiration of higher plants. It is not exactly similar, for Kostychev some years ago found that certain of the higher fungi, such as species of *Agaricus*, failed to form alcohol in the absence of free oxygen. Carbon dioxide, however, was evolved in considerable quantity. When mannite was added to the press-juice it disappeared without the evolution of carbon dioxide. It was possible that the substance producing carbon dioxide here was an intermediate product of oxidation and split off the gas by hydrolysis.

The production of ethyl alcohol by the yeasts is a process of very considerable industrial importance, and its discovery is lost

in the mists of antiquity. Although the main products of the reaction are ethyl alcohol and carbon dioxide, other substances are also produced in varying amounts, such as acetaldehyde, pyruvic acid and glycerol, as well as the two higher alcohols, isobutyl carbinol and secondary butyl carbinol, which are usually spoken of in commerce as amyl alcohol and form the constituents of fusel oil. The presence of fusel oil is especially pronounced in the cheap spirits produced from potatoes.

The hexoses are more suitable for fermentation than the disaccharides as the latter have to undergo a preliminary hydrolysis before they are converted into alcohol and carbon dioxide. Alcoholic fermentation is an enzymic process and the enzyme involved is known as zymase.

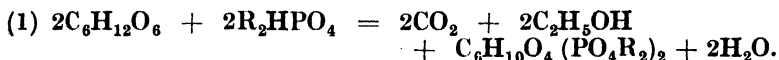
It was early shown by Harden and Young that the zymase obtained from expressed yeast juice is separable into two parts : the enzyme and co-enzyme. The nature of the latter is at present unknown. According to Neuberg and Sandberg (1920), the co-enzyme plays the part of hydrogen acceptor. They were able to show that aldehydes, ketonic acids, nitro-bodies and disulphides can all serve as co-enzymes. Euler and Myrbäck (1924) found that the activity of the co-enzyme could be raised by successive precipitation with lead acetate or even silico-tungstic acid.

### **The Influence of Phosphate in Fermentation**

The addition of alkaline phosphate to the fermenting liquors markedly increases the rate of the reaction, and it was shown by Harden and Young that the increase in rate could be as high as twenty times the original value. In time the rate again falls to the original value and may be increased by a second addition of phosphate.

It was found by Harden and Young that if the solution were boiled, the moment the velocity had fallen to the initial value, the added phosphate could no longer be precipitated by uranium acetate, and that it was in organic union with the hexose as a hexosediphosphate. These workers, therefore, considered that

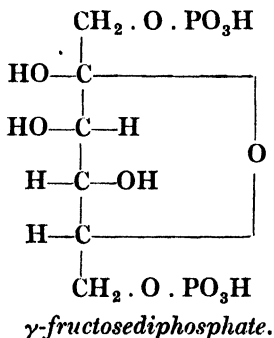
the fermentation takes place in stages and the first part of the reaction consists in the formation of a hexosediphosphate :—



Under the influence of water the hexosediphosphate undergoes hydrolysis to hexose and phosphate once more :—



Whatever may be the nature of the sugar used for fermentation, the hexosediphosphate formed is always the same and is fructose-diphosphate. It is probable that the fructose present in the molecule of the ester is in the  $\gamma$ -form or active form (see Robison and Morgan, 1928).



The matter has since been shown to be very much more complex than was originally considered. Robison (1922) showed that if either glucose or fructose were fermented by yeast, in addition to the hexosediphosphate a hexosemonophosphate was also formed. Harden and Henley (1927) therefore reinvestigated the problem in the light of Robison's discovery. The original equation formulated by Harden and Young was based on the ratio  $CO_2/P$  (total esterified) = 0.9. Harden and Henley found that about 10 per cent. of the phosphorus is esterified without the evolution of carbon dioxide. They considered the product to be a hexosemonophosphate, and that the small deviation from the

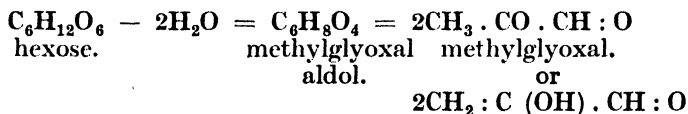
theoretical ratio of  $\text{CO}_2/\text{hexosediphosphate} = 2$  (which they found to be 2.38 on redetermination) required by the original equation could be accounted for by the partial hydrolysis of the hexosediphosphate. This again will not meet the case. Harden and Henley (1929) found that on fermenting glucose and fructose in the presence of phosphate with *dried* yeast, the ratio of extra carbon dioxide evolved to phosphorus esterified, tended to be slightly higher than unity. However, with maceration extract of yeast-juice, the values tended to be lower than unity. Further, with the dried yeast, the ratio of hexosediphosphate to hexosemonophosphate formed in the course of the fermentation was generally high, the product in certain extreme cases being almost entirely diphosphate or monophosphate, and the proportions of these substances varied between 96 per cent. of the former to 86 per cent. of the latter. Yet, in spite of the very great variations shown in the amounts of these products produced, the molecular ratio of  $\text{CO}_2/\text{P}$  (total esterified) was still approximately unity.

These fresh facts make it impossible to express the results by means of the original equation formulated by Harden and Young, and Harden and Henley have now abandoned the suggestion made in their first paper to account for these large deviations from the theoretical ratio. The complexity of the problem of the presence of these phosphatic esters in fermentation is further shown by the fact that, in addition to the hexosemonophosphate isolated by Robison, Robison and Morgan (1928, 1930) have been able to isolate a monophosphatic ester of the disaccharide trehalose. Fermentation of either glucose or fructose gave rise to this compound and it was found in amounts representing up to 5 per cent. of the total phosphorus esterified. The hexosemonophosphate formed in fermentation can be either an aldosemonophosphate or a fructosemonophosphate and can be obtained in maximum yield when fermentation is rapid and there is a considerable excess of inorganic phosphate present. The presence of trehalosemonophosphate increases in amount when fermentation is allowed to continue for some time after the addition of inorganic phosphate, but decreases if fermentation is prolonged for a long period.

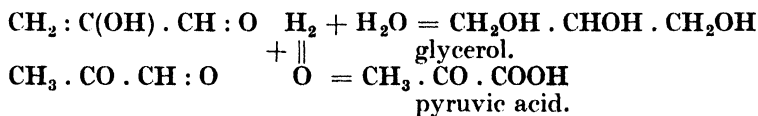
## The Chemical Mechanism of Alcoholic Fermentation

Neuberg and his co-workers have carried out much valuable work in the elucidation of the chemical mechanism of alcoholic fermentation. In the scheme originally promulgated by Neuberg and Kerb (1913) methylglyoxal was the starting-point for the later stages of the reaction. The number of oxidations and reductions involved were all assumed to be brought about by a series of Cannizzaro reactions of the various aldehydes produced as intermediate products.

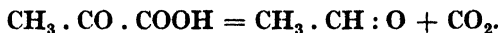
The first stage of the reaction, according to these investigators, consists in the splitting of the sugar into two molecules of methylglyoxal (pyruvic aldehyde), the reaction taking place in two steps :—



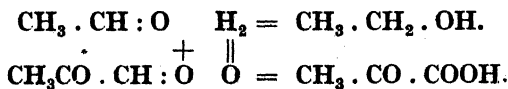
From the two molecules of methylglyoxal by simultaneous oxidation and reduction (Cannizzaro reaction) under the influence of water, pyruvic acid and glycerol are produced :—



By the action of the enzyme carboxylase, which is present in the yeast cells, the pyruvic acid is decomposed into carbon dioxide and acetaldehyde :—

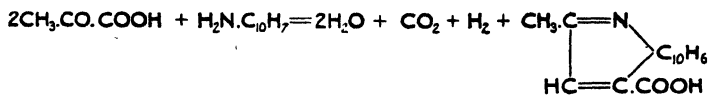


Again under the influence of water, a second Cannizzaro reaction takes place, and the acetaldehyde is reduced to ethyl alcohol with the simultaneous oxidation of a molecule of methylglyoxal to pyruvic acid :—



The pyruvic acid thus formed is converted into acetaldehyde and carbon dioxide.

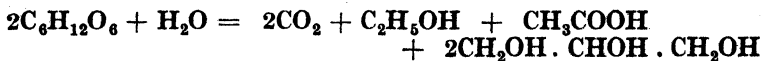
Grab (1921) demonstrated the production of pyruvic acid in alcoholic fermentation by means of the condensation product formed between this acid and  $\beta$ -naphthylamine to give  $\alpha$ -methyl  $\beta$ -naphthocinchoninic acid :—



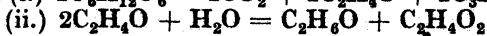
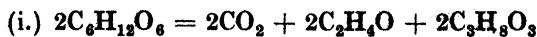
Neuberg and Reinfurth (1918, 1919, 1920), as well as Connstein and Lüdecke (1919) independently, have obtained considerable evidence for the confirmation of this theory of alcoholic fermentation.

Neuberg and his co-workers have investigated the course of fermentation under two different conditions : (a) in the presence of alkaline salts ; (b) in the presence of sodium sulphite.

**Fermentation in the Presence of Alkaline Salts.** It was found by Neuberg and his co-workers that the presence of alkaline salts such as ammonium carbonate and other soluble carbonates and phosphates, markedly influenced the course of fermentation. Provided that the salt were added after the fermentation had become well established, it was found that increased amounts of acetaldehyde, acetic acid and glycerol were produced. It was further discovered that the aldehyde produced was exactly equivalent to the glycerol formed, and Neuberg and Hirsch (1919 ; see also Neuberg, Hirsch and Reinfurth, 1920 ; Neuberg and Ursam, 1920) have shown that this equivalence persisted through the whole course of the fermentation. Neuberg therefore proposed the following equation for this reaction :—



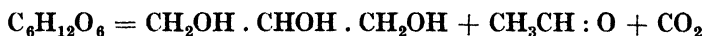
or the reaction may be regarded as proceeding in two steps :—



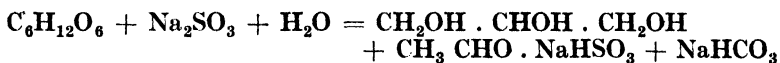
The acetaldehyde produced in the reactions is considered to

undergo the Cannizzaro reaction giving equimolecular proportions of ethyl alcohol and acetic acid, as in equation (ii.).

**Fermentation in the Presence of Sodium Sulphite.** When sodium sulphite was added to the fermenting liquors the amount of alcohol and carbon dioxide diminished. It was also found that the aldehyde produced was exactly equivalent to the glycerol formed (see Neuberg and Hirsch, 1919). Thus the addition of sulphite to the fermenting mixture increases the yield of glycerol. This fact was commercially exploited by the Germans during the Great War for the production of glycerol in large amounts for the manufacture of explosives without fats, animal fats being needed for food. Neuberg proposed the following equation to express this type of fermentation :—



or since sodium sulphite is added to the fermenting liquors the equation can be written :—



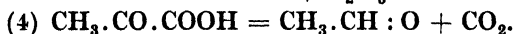
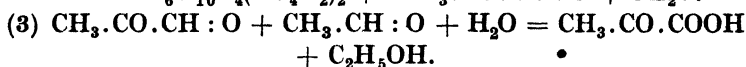
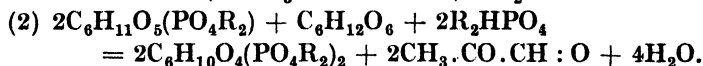
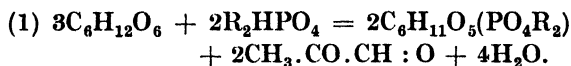
In actual practice it was found that some of the sugar underwent normal fermentation, so that the final result should be expressed by a combination of the equation given above and that usually written for alcoholic fermentation :—



It should be mentioned here that since pyruvic acid,  $\text{CH}_3\text{CO} \cdot \text{COOH}$ , possesses a ketonic group, like acetaldehyde, it can also form a bisulphite compound :  $\text{CH}_3 \cdot \text{C}(\text{OH}) \cdot \text{SO}_3\text{Na} \cdot \text{COOH}$ . Neuberg found that this compound was readily fermented by yeasts, whereas the acetaldehyde bisulphite was not. Thus, only acetaldehyde and not pyruvic acid is fixed in the course of fermentation by the addition of sodium sulphite.

The question arises here as to the connection between the formation of hexosephosphate esters in the initial stage of fermentation and the subsequent chemical reactions postulated by Neuberg and his co-workers. It has already been stated that Neuberg considered the first stage to be a splitting of the hexose

molecule into two molecules of methylglyoxal. On general grounds the rupture of the hexose molecule might be more readily brought about from the active or  $\gamma$ -forms into 3-carbon compounds than from the normal or inactive forms. That the hexosediphosphate formed in the initial stages is a fructosediphosphate has already been described, and it will be recalled that Robison and Morgan thought that the fructose in the molecule is in the  $\gamma$ -form. It has been known for some time that there is a lag period in the evolution of carbon dioxide after the addition of phosphate to yeast preparations, and this is especially the case in maceration extracts of yeast and also in preparations containing much water. The lag period is also greater with glucose than with fructose, but is reduced by the addition of relatively small quantities of fructose. The addition of various hydrogen acceptors, such as methylene blue, pyruvates, and acetaldehyde, much reduce this preliminary induction period. Under almost all conditions the reaction of one molecule of phosphate in fermentation yields one molecule of carbon dioxide, whether monophosphoric or diphosphoric ester be formed. Boyland (1930) has therefore suggested combining Harden's (1930) ideas on fermentation with those of Neuberg, and that the sequence of reactions in the presence of a trace of aldehyde might be represented as follows :—



Of these various reactions the only one likely to be affected by hydrogen acceptors is the oxidation of methylglyoxal to pyruvic acid (equation 3), and as hydrogen acceptors have such a marked effect on the evolution of carbon dioxide, this oxidation is probably the slowest or controlling stage in the beginning of the reaction with phosphate. It should therefore be responsible for the lag in the appearance of carbon dioxide after phosphate esterification.

Boyland showed that this initial lag period is not removed by the presence of hydrogen acceptors, although they cause an increase in the rate of esterification and evolution of carbon dioxide. It was therefore thought that perhaps accumulation of methylglyoxal normally arrests esterification and that removal of methylglyoxal (by oxidation at the expense of hydrogen acceptors) allows of more sugar to become esterified. If this lag in fermentation be due to the relatively slow oxidation of methylglyoxal, then when the lag period is greatest this substance should accumulate. This was found to be the case. Boyland suggested that the action of acetaldehyde in accelerating the reaction of phosphate is probably different from that of dyes like methylene blue. The difference lies in the fact that the reaction is not reversible in the case of acetaldehyde, so that this compound acts solely in the capacity of hydrogen donor and not of hydrogen carrier. The further suggestion is also made that it is probably more effective than dyes in fermentation because of some specificity of the enzyme oxidase for acetaldehyde.

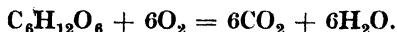
There is now a large amount of evidence to show that acetaldehyde is also a product of anaerobic respiration in the higher plants. Klein and Pirschle (1926) found that acetaldehyde was produced in strongly respiring organs such as buds and seedlings. Similarly, Bodnár, Szepessy and Ferenczy (1925) discovered that peas grown under anaerobic conditions also produce acetaldehyde. If the acetaldehyde were fixed with sodium sulphite the amount of alcohol and carbon dioxide evolved was reduced in amount. Neuberg and Gottschalk (1925) have also discovered that acetaldehyde is a product of anaerobic respiration, and if calcium instead of sodium sulphite were added to the mixture, the quantity of aldehyde was increased and the amount of alcohol reduced.

M. Thomas (1925) has investigated the anaerobic respiration of stored apples. Ethyl alcohol and acetaldehyde were both produced in the course of the experiment, though neither formed the intermediate or end-products of normal respiration in the presence of oxygen. A curious point discovered was that carbon dioxide, even in the presence of oxygen, could cause carbon dioxide zymasis, *i.e.*, respiration was of the anaerobic type. In

such circumstances, the ratio of alcohol to aldehyde was 2 : 1, whereas in anaerobic respiration the ratio was 50 : 1. Hence the presence of a mixture of carbon dioxide and oxygen may be more injurious to the fruits than the entire absence of oxygen.

### The Oxidation Mechanism of the Cell

Respiration is usually defined as the intake of oxygen and the excretion of carbon dioxide from the physiological combustion of carbohydrate and fat, and is expressed by the equation :—



It must be remembered that this equation merely states the beginning and end products of a complex series of reactions. Glucose in ordinary oxygen or air is not appreciably oxidised, and it is therefore obvious that in the living cell some mechanism must be present for activating the oxygen to carry out the oxidation of this carbohydrate at the ordinary temperature.

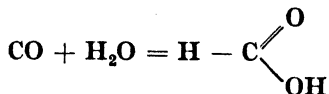
The principal function of respiration is to supply the living organism with sufficient energy to carry out its vital activities, and any oxidation processes occurring in the cell which release usable energy may be classed as respiration. Adopting this wider definition of respiration, a number of different oxidation mechanisms have recently been discovered to be present in the cells of plants and animals, and their nature will now be considered.

The chemical definition of oxidation is more comprehensive than the simple addition of oxygen to a substance. Oxidation, considered from the chemical standpoint, is the addition of oxygen or any electro-negative element to a substance, or the subtraction of hydrogen or any electro-positive element. Similarly, reduction is defined as the subtraction of oxygen or any electro-negative element or the addition of hydrogen or any electro-positive element to a substance.

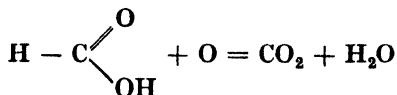
The formation of quinones from phenols is an oxidation reaction involving the subtraction of hydrogen. This type of oxidation seems from recent investigations to be of common occurrence in

living cells. It needs the presence of some substance capable of taking up the hydrogen, which is spoken of as the hydrogen acceptor.

Oxidation is a complex process. Even the oxidation of carbon monoxide to carbon dioxide needs the presence of a minute trace of water, and combination will not take place in the presence of pure, dry oxygen. Wieland has made the suggestion that this reaction takes place in stages. The first stage is the combination of the carbon monoxide and water to give formic acid :—



Under the influence of oxygen the formic acid is oxidised to carbon dioxide and water :—



In the second stage of the reaction, the oxygen plays the rôle of hydrogen acceptor. The number of examples of oxidations brought about by the removal of hydrogen may be multiplied indefinitely, and, according to Wieland, enzymic oxidation reactions are brought about by the activation of the hydrogen of the substrate, and the activated hydrogen is then removed by a suitable hydrogen acceptor such as atmospheric oxygen. He explained such phenomena as the Schardinger reaction, which is used for distinguishing boiled from unboiled milk, as due to the presence of an enzyme which plays the part of a hydrogen acceptor. In the Schardinger reaction, milk is treated with a drop of acetaldehyde and methylene blue. Should the milk have been previously boiled, the methylene blue is not decolorised to the leuco-base on warming ; if the milk has not been boiled, the dye is reduced to the leuco-base. Wieland considered an enzyme to be present, to which the name dehydrogenase, dehydrogenase, or reductase has been given. This enzyme dehydrogenates the aldehyde hydrate first formed and the methylene blue plays the part of hydrogen acceptor.

In physiological experiments the dye methylene blue is much used to detect these oxidation-reduction reactions. In the presence of a hydrogen donator the dye is reduced to the colourless leuco-base, and in the presence of a hydrogen acceptor it is oxidised to the dye once more.

Thunberg (1920) removed, by washing, the substance (glutathione, see below) in frog's muscle which reduces methylene blue. He found that on addition of a variety of organic compounds, *e.g.*, succinic acid, the latter brought about the reduction of the dye, whereas others, such as propionic acid, which does not yield its hydrogen readily, did not. Thunberg considered that the methylene blue was reduced by the giving up of hydrogen by some donator, and that this hydrogen was transported by an enzyme to the acceptor, in this case methylene blue. From his experiments Thunberg suggested that hydrogen is the primary fuel of the cell.

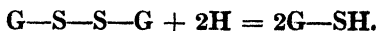
F. G. Hopkins (1921, 1923) isolated from yeast in a non-crystalline form a peptide to which he gave the name *glutathione* which could act either as a hydrogen acceptor or hydrogen donator.

Hopkins showed that it contained glutamic acid and cysteine, and the analytical figures appeared to indicate that it was a dipeptide composed solely of these two amino-acids.

In the presence of a hydrogen acceptor the following reaction was found to take place :—



Whereas in the presence of a hydrogen donator the oxidised glutathione ( $\text{G—S—S—G}$ ) acted as a hydrogen acceptor :—



Like cysteine itself, glutathione in neutral aqueous solution rapidly absorbed oxygen, becoming converted into the oxidised or disulphide form. Hopkins showed that the disulphide form was in turn rapidly reduced back to the sulphydryl form by animal tissues, so that it appeared clear that a portion of the oxygen normally absorbed by the respiring tissues was being utilised by the glutathione system.

Hopkins and M. Dixon (1922) found that freshly-washed muscle does not reduce methylene blue, but on the addition of glutathione in a solution suitably buffered, the power of reducing the dye is restored and enables the system to respire. The washing of the tissue removed the peptide. If the glutathione and washed muscle were heated to 100° C., reduction and respiration still took place. It is clear, therefore, that the glutathione reacts with some thermostable constituent to form a simple and highly efficient autoxidisable system.

Hopkins (1925) discovered that in acid systems of pH 3.0 to 4.5, glutathione facilitated the oxidation of unsaturated fatty acids and lecithin. In neutral or alkaline systems (pH 7.4 to 7.6), the whole nature of the process seemed to be different. While the sulphydryl ( $\text{—SH}$ ) group of the glutathione is being oxidised, the fatty acids are simultaneously oxidised in such a manner that there is an equal division of oxygen between the two. Hopkins also considered that the oxidation of certain proteins by glutathione (either  $\text{G—SH}$  or  $\text{G—S—S—G}$ ) only occurs if the proteins themselves display sulphydryl groups. In the case of the water-extractable proteins of muscle tissue which possess this group, oxidation will only take place in neutral or alkaline media and not if the medium be acid. The sulphydryl group of the protein is oxidised and the total amount of oxygen consumed is ten times the equivalent of the sulphydryl group. The protein can be reduced when the sulphydryl group reappears and with the glutathione a further uptake of oxygen becomes possible, and this uptake is again greatly in excess of the oxygen equivalent of the sulphydryl group.

Allott (1926) showed that the oxidation of fats is by no means so simple as pictured by Hopkins. Different samples of fats give different results, and similar values to those of Hopkins can only be obtained with certain conditions of the oil. For example, the "double-uptake" of oxygen with fatty acids at pH 7.5 seems to depend upon the lack of spontaneous activity of the fatty acid and the iron content of the system, and very possibly other factors may be concerned as well.

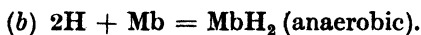
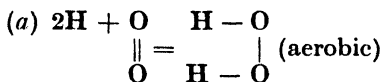
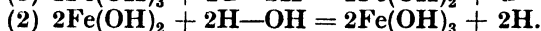
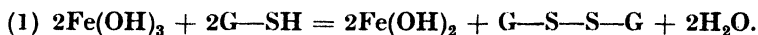
It has been known for a number of years that potassium cyanide

in minute concentrations can stop many oxidations in living cells without effecting any lasting injury, and the respiratory activity is recovered on simply washing with water. Warburg (1914) found that iron was highly important in the respiratory activity of sea-urchin eggs, and that in the absence of iron, respiration came to a standstill. It is therefore probable that the cyanide combines with the iron in the cell and causes the various oxidation processes that are taking place to cease. Since cyanide and iron combine *in vitro* to give complex salts of the type of the ferro- and ferricyanides which are stable bodies, it is difficult to ascribe any explanation of the reverse process brought about by the mere washing of the tissues.

According to Warburg, structure plays an important part in the activity of the cell, in that suitable surfaces are presented for the activity of catalysts taking part in cell reactions. To illustrate this view he has devised his so-called "charcoal-model." He found that if cystine and other amino-acids are shaken with blood charcoal at the ordinary temperature, oxygen is absorbed, and the acids are oxidised to ammonia and carbon dioxide. Iron is found to play an important rôle in these reactions, and narcotics act on the "model" in much the same way as in living cells. Warburg (1923) imagined the cell to be composed of a mosaic of areas of iron and no iron. The cell oxidations are considered to take place on the iron-bearing areas. He has made a number of experiments with blood charcoal containing varying amounts of iron which give strong support to his views. He considered that the function of the iron is that of oxygen carrier or oxygen activator, and that ordinary molecular oxygen is quite unable to act as a hydrogen acceptor unless it is first activated by iron.

Harrison (1924) found that the oxidation of glutathione was markedly accelerated by the presence of small amounts of iron. The iron acted in a catalytic capacity, and potassium cyanide put an end to the reaction. In a later series of experiments, Harrison (1927) was able to carry the matter a stage further, and showed that cyanide affected both the aerobic and the anaerobic phases of the oxidation of glutathione. The anaerobic experiments were carried out in nitrogen. In addition, he discovered that iron

could accelerate both the anaerobic and the aerobic oxidation of glutathione. Such being the case, it is clear that the iron is not acting in the capacity of an oxygen carrier or activator. Harrison made the suggestion that the function of the iron is that of an intermediary catalyst, and that it is alternately oxidised and reduced. Ferric iron is reduced by the sulphhydryl group, and the ferrous salt thus produced reduces the hydrogen acceptor present, which may be methylene blue in the anaerobic and oxygen in the aerobic experiments. In the latter case, hydrogen peroxide will be produced and the iron is simultaneously oxidised back to the ferric state. The ferric iron is then reduced by a further quantity of glutathione :—



Although it was thought by Hopkins that glutathione was a dipeptide, Hunter and Eagles (1927) made the persistent claim that the glutathione they were able to isolate from yeast, liver, and blood, by the method originally described by Hopkins, always contained less than the theoretical percentage of sulphur required by diglutaminyl cystine (9.37 per cent., as against 12.85 per cent.), and although they found both cysteine and glutamic acid to be present, they considered from their analytical findings that something additional was also present as well. As a result of this work, Hopkins (1929) was led to reinvestigate the composition of glutathione, and he has now shown it to be a tripeptide of cysteine, glutamic acid and glycine. The compound was eventually isolated in the crystalline condition *via* the copper salt. Kendall, Mason and McKenzie (1930a, 1930b) have since shown the constitution of the tripeptide to be :—

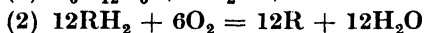
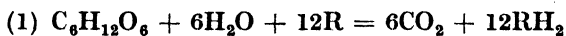


*γ*-glutamyl-cysteinyl-glycine.

Meldrum and M. Dixon (1930) have reinvestigated the catalysis of oxidations by glutathione, using the pure crystalline material. The pure crystalline product, like the older preparations, was found to take up oxygen, and its oxidation was strongly inhibited by M/1000 and completely prevented by M/100 KCN. The oxidation is therefore presumably due to the presence of traces of catalytic metals, as in the case of cysteine. On the other hand, the pure product differed from the impure, inasmuch as the addition of traces of iron and copper salt did not accelerate the uptake of oxygen to any marked extent. In the circumstances, the presence of catalytic metal cannot be the limiting factor in the oxidation of the new tripeptide preparations. The new crystalline product was found to be reduced by the addition of muscle powder, but, unlike the old product, there was no uptake of oxygen. It follows from this that the pure glutathione is unable to bring about oxidation of proteins. Further, since the tissue can reduce glutathione, it follows that reduced glutathione itself cannot oxidise in the presence of muscle tissue. As far as the oxidation of fats is concerned, there is no difference in the behaviour of the new and old preparations. It is suggested by Meldrum and Dixon that, although the oxidation of glutathione is due to catalysis by metals, the slowness of the oxidation of the crystalline preparations is not due to any lack of catalytic metals and the rate of oxidation must be determined by some other factor. "The facts seem most readily explained by the assumption that the active catalyst is not the metal itself but a complex of the metal with some other substance present in traces in the glutathione."

It will be necessary at this stage to consider briefly Palladin's views on the respiration mechanism of plants. He considered that certain chromogens, to which he gave the name "respiratory chromogens," were widely distributed in the plant world. To obtain these chromogens all that is necessary is to boil the tissues with water and filter the liquid. Peroxidase (see below) and hydrogen peroxide are then added, when a red colour is developed by the formation of the respiration pigment from the oxidation of the chromogen. This, by further oxidation, is converted into a black or violet-black substance. Palladin supposed that respira-

tory chromogens were present in the form of pro-chromogens, which were in the nature of glucosides, and these pro-chromogens were oxidised by the enzymes present in the cells to chromogens. By forming water, chromogens remove hydrogen produced in respiration. According to Palladin all carbon dioxide formed in respiration is anaerobic in origin and the respiration chromogens are reduced by substances formed during anaerobiosis. Subsequent oxidation leads to the formation of the pigment once more :



Equation (1) represents the anaerobic stage of the process and (2) the aerobic phase. The chief criticism that can be levelled at this theory is the fact that in a large number of instances, oxidase systems are absent in higher plants, so that this type of reaction cannot be of universal occurrence.

P. Haas and Hill (1925a, 1925b, 1926) have been able to extract a colourless chromogen from the leaves of *Mercurialis perennis*, which shows a marked avidity for oxygen. To this substance they have given the name hermidon. Hermidon is capable of undergoing oxidation in two stages, yielding first a fugitive blue compound, cyanohermidon, and in the second stage a stable yellow pigment, chrysohermidon. The volume of oxygen fixed at each stage of the oxidation is the same. The reaction is reversible. When treated with sodium hydrosulphite the blue cyanohermidon is reduced to hermidon, while, on shaking, with air the colour is restored. The reduction of the yellow compound can only be brought about by the aid of fairly drastic reducing agents, such as the aluminium-mercury couple, which may also be used for the reduction of the cyanohermidon. It was also discovered that the leaves of the plant, soaked in a vessel containing cyanohermidon, reduce the latter to hermidon, but fail to reduce chrysohermidon.

Haas and Hill suggested that hermidon plays a part in the respiration of *M. perennis*, since it is most abundant when the respiration of this plant is at its highest in spring and summer. These authors considered that the hermidon suffers oxidation to

cyanohermidon and the cyanohermidon is reduced to hermidon once more, losing its oxygen to some acceptor or reducing agent which may be of the nature of a metabolite. Cannon (1926) has studied the electrode potentials of the system hermidon-cyanohermidon over a wide range of  $pH$  (2.0 to 8.0), and the system cyanohermidon-chrysohermidon over the range of  $pH$  7.0 to 8.0. The second system was found to be reversible only in the neighbourhood of neutrality, as the line-oxidant was subject to an irreversible non-oxidative change, the velocity of which was a function of the hydrogen-ion concentration.

W. A. Roach (1925) has also found a blue pigment in slightly sprouted potatoes which had been placed overnight in an atmosphere of carbon dioxide in a vacuum desiccator and later pulped in an atmosphere of this gas. The filtrate from the pulp was of an opalescent blue colour tinged with green. On the admission of air, the blue colour became more intense, and with still further exposure it changed through various shades of green to light yellow. From the yellow, still further colour changes occurred, till finally a brown-black was formed. The blue shade was discharged on the addition of sodium hydrosulphite. It would seem that the potato, like *Mercurialis perennis*, contains a compound resembling hermidon, or this compound may even be generically related to hermidon.

Quastel (1926), from his studies on resting bacteria, has brought forward an interpretation of oxidations and reductions *in vivo* which embraces the views of Wieland, Thunberg and others. The bacterium employed was *Bacillus coli*. Of 108 substances tested, 56 were found to be hydrogen donators or acceptors. If enzymes were responsible for the transport of hydrogen from donator to acceptor, it is in the highest degree improbable that there are 56 specific enzymes dealing with one type of phenomenon; moreover, all attempts to isolate such enzymes have failed. Even were enzymes responsible, the actual mechanism of activation still remains to be elucidated. The problem of determining the mechanism therefore resolves itself into three separate problems:

- (1) determination of the site of activation of a substrate molecule;
- (2) determination of mechanism of activation of this molecule;

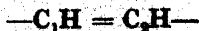
and (8) determination of process of oxidation after activation has occurred.

From a consideration of the experimental data, Quastel considered that the site of oxidation of the substrate molecule is at the cell surface. In all growing organisms we have a series of co-ordinated chemical reactions taking place. If the reactions were not co-ordinated there would be no growth or other vital activity. The ordered behaviour of chromosomes shows this to be so. In other words, an ordered series of events must be taking place in the cell.

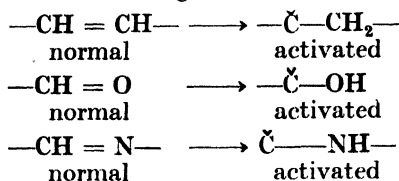
The work of Hardy, Langmuir and others has shown that membranes have a definite structure. The molecules composing the membrane are definitely orientated with regard to one another in a particular way dependent upon their chemical nature and the character of the phases on either side of the membrane. From its complex nature it cannot be expected that the membrane possesses a homogeneous character or even shows symmetry. Certain parts would be occupied by certain molecules or groups of molecules, and other parts by other molecules or groups of molecules. It is probable that there is an irregular distribution of such groups, *i.e.*, they have a geography.

Now, associated with certain groups of molecules orientated in the membrane, there will exist electrical fields, the nature of which will depend on the nature of the groups. Some will be very powerful and others weak, *i.e.*, they will show varying intensity.

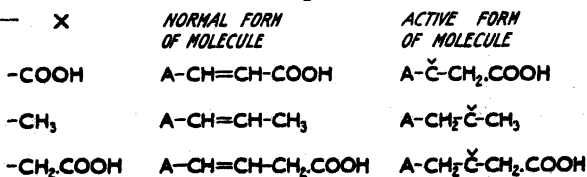
**Mechanism of Activation.** If the effect of an external electric field be considered upon an unsaturated linkage, say  $C_2 = C_1$ , the octets of electrons round the carbon atoms,  $C_1$  and  $C_2$ , will have four in common. If the external electric field act from left to right on the molecule, some of the electrons held in common may be so far displaced from  $C_2$  to  $C_1$  that they can no longer be considered as being held in common with  $C_2$ . If two were to go, then  $C_1$  has a complete octet and  $C_2$  is reduced to a sextet. Thus  $C_1$  has become saturated and  $C_2$  unsaturated and chemically more active. The same force which alters the electrons will also alter the relative positions of the protons (H-ions), thus in :—



the polarising field upon the double bond will cause a shift of proton to  $C_2$  or *vice versa*, the extent of the shift depending on the strength of the external field. The greater the shift, the more unsaturated and chemically active will one end of the bond become. The following equations represent the change after maximum activation :—



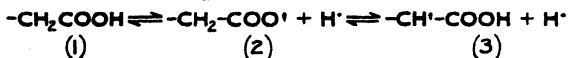
A second factor that must now be considered is that not only will there be the external electric field, but the electrical condition of the molecule must also be taken into account in determining the actual shift of the proton. It has been shown by Thomson that the electrical effect of replacing an atom of hydrogen by another atom or radical may be represented by the introduction of an electric doublet at the hydrogen atom. So that if the radical form a system requiring an electron to complete the octet, the positive end of the doublet will be towards the molecule with which the radical is combined. Thus, if a radical replaces a hydrogen atom combined to a carbon atom, the positive end of the doublet will be towards the carbon and the electrical effect at the carbon atom will be to drive away positive and attract negative electricity. The reverse effect holds with a radical in which there is one electron over, after providing for complete octets. Take, for example, the structure  $A\text{—CH} = \text{CH—X}$ , where A is assumed to have no directive influence on the movement of the proton. If maximum activation has taken place, the following results can occur :—



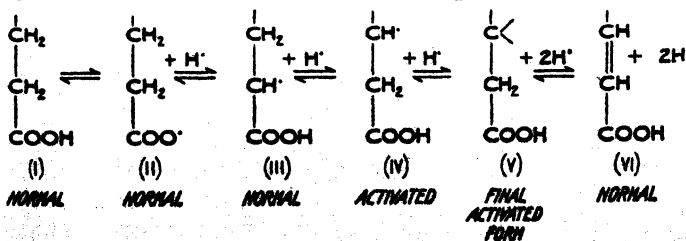
The effect of the carboxyl group at X is to produce an electric field at the  $\alpha$ -carbon atom which attracts positive electricity. It

will also affect the  $\beta$ -carbon atom to some extent, and may even render this more liable to attract positive electricity than it was before the introduction of the carboxyl group. The field at the  $\alpha$ -carbon atom, however, is very much stronger, and there will be a shift of proton from the  $\beta$ - to the  $\alpha$ -carbon atom. The  $\beta$ -carbon atom will be now unsaturated and the  $\alpha$ -atom saturated. Hence, when oxidation occurs *after* activation, an asymmetrical oxidation is impossible, and only  $\beta$ -oxidation will take place. When the methyl radical is in position X there will be a passage of proton from  $\alpha$ - to the  $\beta$ -carbon atom after activation and  $\alpha$ -oxidation will occur.

**Saturated Bond Oxidation.** In the case of the oxidation of saturated compounds, different conditions arise. Take, for example, a compound of the type  $-\text{CH}_2-\text{CH}_2-\text{COOH}$ . Here the condition of the carboxyl group has to be considered in detail. The charged carboxyl group has an effect on the  $\alpha$ -carbon atom, the hydrogen will be rendered more mobile and will oscillate between the  $\alpha$ -carbon atom and one of the oxygen atoms of the carboxyl group. Thus the normal forms of the structure,  $-\text{CH}_2 \cdot \text{COOH}$ , will be represented by the equilibria:—



The movement of the  $\alpha$ -H(proton) is more likely in a saturated than in an unsaturated structure, *i.e.*, in  $-\text{CH}_2 \cdot \text{COOH}$  than in  $=\text{CH} \cdot \text{COOH}$ ; for in the latter the attractive effect of the  $\alpha$ -carbon atom for positive electricity will oppose the attractive effect of the oxygen of the carboxyl group. If, however, there be attached to the  $\alpha$ -carbon atom a group which has the effect of repelling positive electricity at this atom, a shift or movement of hydrogen will take place, so that the activation of a saturated group such as  $-\text{CH}_2-\text{CH}_2 \cdot \text{COOH}$  will proceed as follows:—

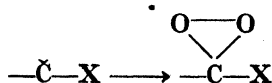


On activating (III) proton will move from the  $\beta$ - to the  $\alpha$ -carbon atom, *i.e.*, towards the atom with the strongest positive electricity. Now (IV) will also undergo the same process, and the final activated form will be (V). But this is simply the activated form of (VI). So that, if there be in the system an activating source, the structure (I) and a hydrogen acceptor, the affinity of which for hydrogen is greater than that of (V), a reaction will take place to produce (VI). This scheme,  $I \longrightarrow VI + 2H$ , represents in the clearest way Wieland's theory of the activation of hydrogen.

For the mechanism of oxidation of dibasic acids, substituted acids, and other compounds, the original paper should be consulted.

A further point requires consideration here. Hydrogen acceptors fall into two classes : (i.) those that require activation by the cell (or the oxidising power of which is increased by the cell) ; and (ii.) those hydrogen acceptors the oxidising power of which is independent of the cell. To the former belong nitrates, formates and chlorates, and in the latter methylene blue.

There are three ways in which oxygen may be activated : (a) by iron (Warburg), (b) sulphhydryl group (Hopkins), and (c) substances capable of peroxide formation, *i.e.*, those substances containing the group :  $-\dot{C}H=CH-$ ,  $-\dot{C}H=O$ , and  $-\dot{C}H=N-$ . These become activated to  $-\dot{C}-CH_2-$ , etc. It seems possible, therefore, that oxygen may form a temporary link with the activated structure at the point of the unsaturated carbon atom :—



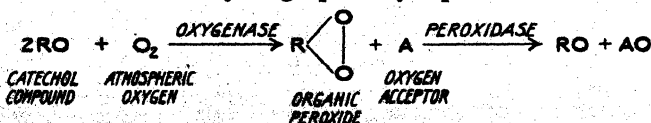
This compound having high oxidising power, and being in effect "active" oxygen, the very process which activates the hydrogen of the donator may also result in activating the oxygen. From the views put forward in this paper it will be seen that it is totally unnecessary to postulate the existence of numerous specific enzymes dealing with oxidations and reductions, but that it is possible to regard specificity of behaviour as belonging rather to the molecules themselves than to the enzymes.

### Enzymes concerned in Oxidation

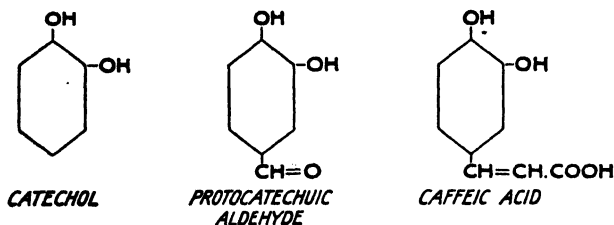
A number of enzymes are known in plant cells which are directly concerned with oxidation processes. The most important of these are : oxidase, peroxidase, zymase, carboxylase, tyrosinase and catalase.

**Oxidase and Peroxidase.** Oxidase is capable of carrying out a number of oxidations in the presence of free oxygen. Freshly prepared 1 per cent. alcoholic solution of tincture of guaiacum is a delicate test for the presence of oxidase, when a deep blue colour is developed. If tincture of guaiacum and hydrogen peroxide are brought into contact with certain tissues such as horse-radish, a blue colour is formed, but no colour is given without the addition of hydrogen peroxide as well. This result is due to the presence of a peroxidase. It is only in the presence of a peroxide that a peroxidase is capable of carrying out its oxidative reactions, releasing oxygen from the peroxide. Thus guaiaconic acid, which is found in tincture of guaiacum and gives the blue oxidation product mentioned above with active oxygen, is stable to hydrogen peroxide alone, but on the addition of the peroxidase it is oxidised to the blue substance. According to the view originally propounded by Bach and Chodat, the oxidase system which acts directly on tincture of guaiacum is composed of two enzymes, peroxidase and oxygenase, and the function of the latter is to activate the oxygen of the air to form the necessary peroxide.

Wheldale Onslow (1919, 1920) has found that various tissues which give the direct oxidase reaction with tincture of guaiacum contain catechol derivatives. When solutions of these dihydroxy-phenols are exposed to air, they slowly autoxidise, giving rise to brown oxidation products. These brownish products are accompanied by the formation of a peroxide, either an organic peroxide or hydrogen peroxide itself. In plants this reaction is catalysed by oxygenase. Thus, according to Onslow, the complete oxidase system consists of oxygenase, peroxidase and catechol compound, and their interaction may be graphically represented as follows :—



If the catechol compound be extracted with alcohol, the formation of the peroxide of the system is prevented and the peroxidase is left in the tissue as a residue. On the addition of a little catechol, however, the system is once more reformed. Tissues which do not brown on exposure to air are said not to contain the catechol component. The most important of these catechol derivatives are catechol itself, protocatechuic aldehyde and caffeic acid. Derivatives of these substances are widely distributed in the plant world :—



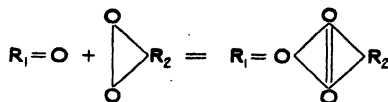
These results of Onslow have been criticised by Gallagher (1923), who considered that the peroxide present in plant tissues arises from some autoxidisable substance. One such substance that was isolated from plant tissues by this investigator was supposed to bear some resemblance to the lipins. Thus oxygenase, according to Gallagher, is an autoxidisable lecithin-like substance. Onslow's results, such as the blackening of tissues on injury, are said to be due, not to the presence of a catechol derivative, but to the action of tyrosinase on tyrosine to give the black pigment melanin. The positive reactions obtained by Onslow for catechol derivatives with ferric chloride are ascribed to the presence of tannins.

Gallagher (1924) visualised oxidation in the living cell by supposing that the lipin-like oxygenase is autoxidised to a super-

oxide of the type  $R \begin{array}{c} \diagup O \\ | \\ \diagdown O \end{array}$ . This superoxide is considered to be

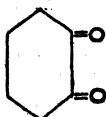
organic in nature and to be derived from an aldehyde. It can fully recover its powers, temporarily lost by boiling, when allowed to stand after cooling. Iron was found to be present in the author's preparations of peroxidase, and since iron salts are known to activate the peroxidase activities of aqueous solutions

of aliphatic aldehydes—which already exhibit peroxidase functions when alone—he deduced that an aldehyde is the precursor or zymogen of the peroxidase, which is oxidised under the catalytic influence of iron to a substance of the type  $R = O$ . The mode of oxidation of such a system is supposed to consist in the combination of the two oxides with the production of a compound with an oxidation potential comparable to ozone, and higher than that of either of the components of the reaction ; this new substance is the actual oxidising agent :—

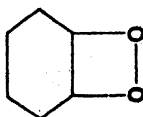


The number of postulations and assumptions made by Gallagher is too large to allow of any great credence being placed on the results. M. E. R. Robinson (1924) repeated Onslow's work, and has confirmed it in most particulars, and has also found that the oxidase of the basidiomycetes differs fundamentally from that of the higher plants and is composed of an enzyme-like peroxide and peroxidase. The lecithin-like substance described by Gallagher was isolated but only gave the peroxide reaction after nineteen days, with horse-radish and tincture of guaiacum. This is certainly too long a period to allow of any connection of this substance with the active oxidations of the living cell.

Recently Szent-Györgyi (1925) has made the suggestion that there is no need to postulate the existence of a peroxidase in the direct-oxidase system. In his opinion, the only necessities for the direct acting oxidase system, which blues guaiacum resin without the presence of hydrogen peroxide, is an oxidase and a substrate containing a catechol derivative. By the action of the oxidase the catechol compound is converted into an *o*-quinone. These *o*-quinones give a blue colour with guaiacum tincture without the intervention of an enzyme. There are two possible types of *o*-quinones :—

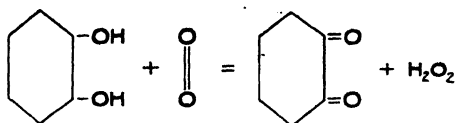


I. STABLE



II. UNSTABLE

Szent-Györgyi considered that peroxidases are merely attenuated forms of oxidase and are not specific enzymes. The presence of fully authenticated peroxidases in certain tissues is due, in his opinion, to the absence of catechol compounds; these substances being replaced by hydrogen peroxide or to a hydroquinone substrate which is oxidised to a *p*-quinone. These results have in general been confirmed by Onslow and M. E. R. Robinson (1926), who put forward the view that molecular oxygen acts as hydrogen acceptor in the reaction, oxidising the phenolic compound to a quinone, and at the same time forming hydrogen peroxide :—



They have been able to detect hydrogen peroxide qualitatively among the reaction products. In this particular case the so-called oxygenase must play the *rôle* of a dehydrogenase. They were quite unable to bring about the oxidation of catechol anaerobically in the presence of methylene blue, so that there is no evidence that oxygenase can act in this capacity.

The nature of the peroxidase in horse-radish has been investigated by Willstätter and Stoll (1918) and Willstätter and Pollinger (1923). They were able to obtain a very active preparation by leaving the roots in running water to remove dialysable impurities. The roots were then treated with oxalic acid which precipitated the enzyme upon protein. The peroxidase and protein were then separated by treatment with alkali and successive adsorption on kaolin, which removed carbohydrate impurities as well as a glucoside. Following this treatment, the preparation was adsorbed on alumina, precipitated with tannin, reabsorbed on alumina and precipitated with alcohol. The view originally expressed by Willstätter was that the enzyme was of the nature of a nitrogenous glucoside, containing over 80 per cent. of pentose and an equimolecular proportion of glucose and iron. The first experiments with this preparation appeared to show a correlation of activity with the iron content, but later experiments failed to

show any such connection. In still more purified samples, neither protein nor carbohydrate was discovered, and the iron percentage was reduced to 0.06 per cent. It would thus seem that the iron is an impurity. On the other hand, the new and purer preparations still contained from 9.37 to 13.57 per cent. of nitrogen.

In connection with peroxidase and its reactions, Keilin (1925, 1926, 1929) has described a thermostable respiratory pigment to which he has given the name *cytochrome*. Cytochrome is widely distributed in the animal world and has also been shown to be present in plants. It occurs in bakers', but not brewers' yeast, and also in bacteria. With tincture of guaiacum and hydrogen peroxide, it gives the peroxidase reaction.

The oxidation and reduction of the pigment can be easily seen in yeast. In the reduced form it shows a clear absorption spectrum with four bands: *a*-6046, *b*-5665, *c*-5502, *d*-5210 Å. In the oxidised form of the pigment there are no clear absorption bands, but only a faint shading from 520-540/550-570. If a shallow tube (30 mm. high) be half-filled with bakers' yeast in water (20 per cent.) and the suspension examined with a Zeiss micro-spectroscope, the four absorption bands can be easily seen, but when air is rapidly bubbled through the suspension, the cytochrome becomes oxidised and the bands disappear. If the current of air be stopped, the pigment becomes reduced, and the four bands gradually reappear.

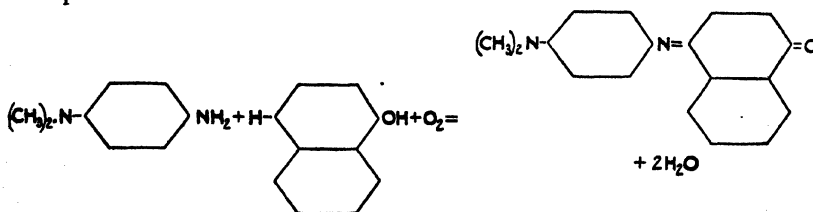
Potassium cyanide (N/10,000) has a narcotic effect and the cytochrome remains reduced. If a drop of potassium cyanide be added to a yeast suspension, kept at low temperature, and previously oxidised by a current of air, the cytochrome becomes immediately reduced, as though the cyanide were acting as a powerful reducing agent. Sodium pyrophosphate has an effect similar to the cyanide. On the other hand, substances such as formaldehyde, acetone, and ethylurethane which kill yeast, do not inhibit oxidation but stop reduction, and the cytochrome remains permanently oxidised.

In reality the yeast cells contain four hæmatin compounds; an unbound protohæmatin and three hæmatin compounds, *a'*, *b'*, *c'* of cytochrome, and these are capable of being oxidised and

reduced independently of each other. The three cytochromes,  $a'$ ,  $b'$  and  $c'$ , are considered to be formed from the free intracellular protohæmatin which is present in all aerobic cells.

Keilin considered that hæmatin compounds are responsible for the peroxidase reactions of bacteria. Thus the aerobic bacteria (*Bacillus subtilis*, *B. proteus*, *B. megatherium* and others) contain a thermostable peroxidase and are rich in cytochrome, whereas the anaerobic bacteria (*B. sporogenes*, *Streptococcus acidilactici*), which do not give a peroxidase reaction, are completely devoid of hæmatin compounds. Of the four hæmatin compounds, the compounds  $a'$ ,  $c'$  of cytochrome are not autoxidisable, while  $b'$  and the unbound protohæmatin are autoxidisable, and the latter in the reduced state will combine with carbon monoxide.

In addition to their thermostable peroxidase system, Keilin, contrary to the results of Harden and Zilva (1914), has also been able to show that yeast cells contain a powerful thermolabile oxidase system, which rapidly oxidises *p*-phenylenediamine to a dark purple quinonoid compound, and also reacts with the Nadi reagent, *p*-aminodimethylaniline and  $\alpha$ -naphthol giving indophenol blue :—



This oxidase is irreversibly destroyed at 70° C., and is markedly inhibited in its action by potassium cyanide and hydrogen sulphide, though sodium pyrophosphate has no effect upon its activity. All the factors which inhibit the activity of this oxidase or destroy it completely affect in the same way the oxygen uptake of the cells, which demonstrates that this "indophenol oxidase" plays an important part in cellular respiration.

This oxidase is responsible for the oxidation of cytochrome, especially of the portions  $a'$  and  $c'$  which are non-autoxidisable, since the oxidation of cytochrome is abolished, or at any rate

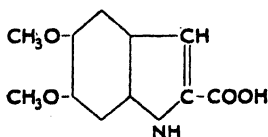
inhibited by the same factors which inhibit or abolish the activity of indophenol oxidase. It was discovered that the reduction of cytochrome could be effected by a number of organic compounds such as sodium succinate, lactate and pyruvate; of these, lactate was the most efficient. Keilin considered that these are first activated by dehydrase, so as to become donators of hydrogen. Thus, cytochrome acts as a carrier between two types of activating mechanisms in the cell: (i.) the dehydrases, activating the hydrogen of organic molecules; and (ii.) the indophenol oxidase activating oxygen. In other words, the cytochrome acts as a hydrogen acceptor which is specifically oxidised by the indophenol oxidase. Harrison (1929), who has made an examination of the indophenol reaction, considered that there is no need to postulate a specific indophenol oxidase to bring about the oxidation of either cytochrome or indophenol. According to this investigator, either glutathione or some other hydrogen donator is involved in the process and in the presence of molecular oxygen is oxidised by subtraction of hydrogen and the formation of hydrogen peroxide. The hydrogen peroxide, in its turn, is reduced by peroxidase to water and the reduced cytochrome oxidised.

**Tyrosinase.** Tyrosinase oxidises monohydric phenols, their derivatives and the amino-acid tyrosine, giving the black pigment melanin. It is present in many fungi, especially *Russula*. It has also been found in wheat bran and in the peripheral regions of the potato tuber near the skin.

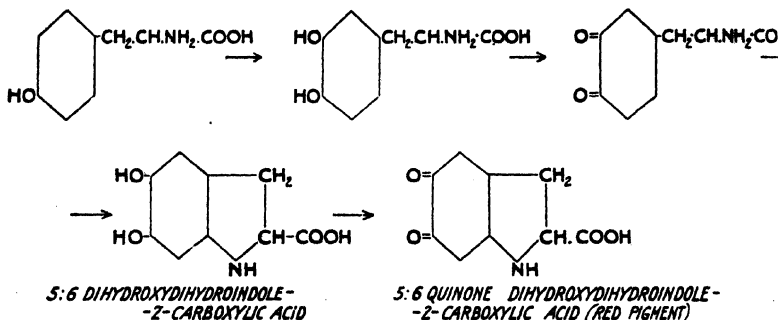
It was shown by Raper and Wormall (1923) that the first visible product, when tyrosine is oxidised by tyrosinase, is a red pigment. This is a very unstable body and spontaneously changes to a colourless substance, which in turn suffers oxidation in the air to the black pigment melanin. In the course of these reactions no deamination of the tyrosine takes place.

The reactions involved in these various changes have been very fully investigated by Raper (1927). It was found that if the red compound were allowed to decolorise *in vacuo* or in the presence of sulphurous acid and then concentrated in an atmosphere of carbon dioxide, it methylated with methyl sulphate to give two crystalline products, one an acid and the other a feeble base.

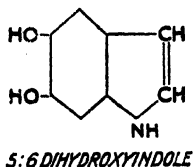
These were found to be indole derivatives. The acid had the structure of a 5 : 6-dimethoxy-indole-2-carboxylic acid :—



The changes to the red pigment are therefore represented as follows :—



The red pigment suffers slow auto-reduction with loss of carbon dioxide to give the colourless body which has the structure :—



and this, in the presence of atmospheric oxygen, is oxidised to melanin.

**Carboxylase.** The enzyme carboxylase removes carbon dioxide from the carboxyl group of ketonic acids giving the corresponding aldehyde. Its function in alcoholic fermentation has already been considered and the matter will not be discussed further here.

**Catalase.** This enzyme is widely distributed in the plant

kingdom and decomposes hydrogen peroxide into water and molecular oxygen. Its function is obscure, but it would appear in many cases to play a protective rôle. Loew has shown that in the oxidation of the purine bases by the xanthine oxidase, the enzyme is destroyed by the gradual accumulation of hydrogen peroxide. If, however, catalase be present, the oxidase remains unharmed, as the hydrogen peroxide formed in the reaction is quickly decomposed to water and oxygen. Loew on this account suggested that catalase plays a protective function in living organisms.

The exact function of catalase in germination has been much investigated in recent years, but the problem still remains to be solved. According to Gračanin (1926), the activity of the catalase of germinating seeds generally increases to a maximum in about four or five days. After the fourth day there is a decrease in catalase content of the cotyledons, but an increase of the enzyme in the root to a constant value. In the fully developed plant the catalase is chiefly found in the leaves and roots. He also found that the catalase is chiefly located in the embryos of dicotyledonous seedlings, whereas in monocotyledons, such as maize, it is present in both embryo and endosperm and in small amount in the testa. From its presence in these structures, it presumably exercises some function in germination.

Morinaga (1925) found that the catalase content of dry rice is only one-tenth that of barley, oats, wheat or rye. Rice normally germinates under conditions in which free oxygen is not abundant. On the other hand, if rice be allowed to germinate under aerobic conditions, the catalase content rises to the same value as in barley, oats, wheat, and rye. Under anaerobic conditions, the catalase content of rice does not increase, but in a medium of low oxygen content there is a slow increase during germination. Hence the ratio of catalase increase is a function of the free oxygen of the medium.

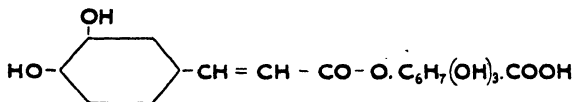
Burge and Burge (1924) found that a fall in temperature produced a decrease in the catalase content of *Spirogyra*, and a rise in temperature an increase of catalase. This is in keeping with the fact that a fall in temperature decreases the catabolic

activity of the plant, and that a rise increases it. Light was also found to increase the catalase content of *Spirogyra*, but its action was less effective than temperature.

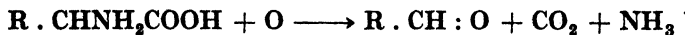
The reaction of the medium has an important influence on the activity of catalase. It is quickly destroyed in acid media.

### The Oxidation of Fats and Proteins in the Plant

Little is known about the oxidation of fats and proteins in the plant cell. Oparin (1921, 1927) has investigated a depside, chlorogenic acid which is very widely distributed in the plant world, although, strangely enough, it does not occur among lichens, which form the chief source of most other depsides. This chlorogenic acid is optically active, is not precipitated by gelatin and gives a green colour with ferric chloride. On hydrolysis with mineral acids it gives rise to caffeic acid and quinic acid, and its constitution may accordingly be written :—



According to Oparin, chlorogenic acid is easily oxidised by atmospheric oxygen with loss of four atoms of hydrogen giving a green pigment. This pigment is capable of acting as a hydrogen acceptor, and can play the part of an oxidising agent. The calcium salt of the fully oxidised acid has the formula  $\text{C}_{32}\text{H}_{32}\text{O}_{19}\text{Ca} \cdot 2\text{H}_2\text{O}$ , and the reduced salt of the acid the formula  $\text{C}_{32}\text{H}_{36}\text{O}_{19}\text{Ca} \cdot 2\text{H}_2\text{O}$ . Chlorogenic acid is an active oxidising agent for  $\alpha$ -amino-acids, peptides and peptones, giving rise to carbon dioxide, ammonia and aldehyde :—



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## CHAPTER VII

### GROWTH

*General—The Nature of Growth Curves—Temperature—Electricity—Climatic Factors—The Frost Resistance of Plants—Carbohydrate/Nitrogen Ratio.*

#### General

AIDED by assimilation and respiration, the seedling or sporeling is transformed into the mature individual. Growth is the final expression of successful metabolism. Without metabolism there can be no growth, and when metabolism ceases, death supervenes. It was early shown by Sachs that the only true criterion of growth is increase in dry-weight (material dried to constant weight at 100° C.), for transpiration and water absorption lead to considerable variations in fresh-weight, and no reliance can be placed on such figures. Estimation of dry-weight, however, has the disadvantage that the plant is killed, and now increase in leaf area is much used to measure growth, for it has been shown that increase in this organ is also an index of true growth (see below).

Both the anabolic and the catabolic sides of metabolism are necessary for growth. The living plant must be supplied with food, and it must also be supplied with energy, without which its vital activities would soon cease. It is necessary, however, that the anabolic side of metabolism should be in excess of the catabolic, or no balance of material would be available for growth.

It has been shown by Boysen-Jensen (1918) that a balance is always available under ordinary conditions for growth. He attempted to elucidate the economic working of two biologically different types of plants: *Sinapis*, a sun-loving plant, and *Oxalis*, a shade type. In *Sinapis* the maximum intensity of assimilation was estimated at 6 mg. of carbon dioxide per 50 cm.<sup>2</sup>

of leaf area every hour at 20° C. The dry-weight of a plant with a leaf area of 50 cm.<sup>2</sup> was 0.279 gm., and the respiratory rate was found to be 0.8 mg. of carbon dioxide for the same leaf area and at the same temperature. Boysen-Jensen was therefore able to calculate that the amount of dry-matter assimilated in the course of a day was 60 mg., and loss in dry-matter from respiration as 14 mg., or, in other words, the gain of material over loss was 46 mg., or 16.5 per cent. Here there is an ample margin of the anabolic over the catabolic side of metabolism to allow of fresh growth. In shade plants, such as *Oxalis*, the assimilation and respiration values are considerably lower than in *Sinapis*. In *Oxalis* itself the maximal assimilation was 0.8 mg. of carbon dioxide per 50 cm.<sup>2</sup> of leaf area every hour at 20° C., while the respiration rate varied between 0.1 to 0.2 mg. for a similar leaf area for the same time and temperature. Again, it is evident that there is a balance of material available for growth.

External and internal factors have a powerful influence on the growth of the living plant. If the factors are not suitable, growth may be retarded or even completely inhibited; or again, the absence of one or more factors may act in a detrimental manner on the well-being of the plant. The influence of some of these factors on growth will be discussed in the following pages.

### The Nature of Growth Curves

A number of attempts have been made within recent years to give mathematical expression to the growth curves of plants. It was shown many years ago by Sachs, that in general terms the growth of a plant could be expressed as an S-shaped curve. The growth rate began slowly, and was followed by a rapid period of increase, the so-called "grand-period of growth," and this period of rapid increase gradually fell away.

Fungal hyphae grow rapidly at first, and then fall into a rate dependent on the medium, temperature, and the nature of the fungus. The extension in growth of fungal hyphae occurs exclusively at the tip. Henderson Smith (1924) found that if the

distance were measured between the tip and some recognisable point on a hypha of *Botrytis cinerea*, the rate of growth was at first slow and gradually increased to a maximum, at which it remained constant for a time; branch hyphæ showed a similar increased rate of elongation. If the whole hyphal system (parent and branches) were taken as a unit, then the rate of growth of the whole system remained constant for many hours. This latter result was said to be due to the difficulty of passing food forward quickly enough to the actively growing hyphal tip. The concentration of the food was no longer the same, and a pressure gradient was established from the tip backwards. The tip, therefore, though it increased in length, no longer advanced at a rate proportionate to the whole length of the hypha, but fell more and more behind that rate.

In the growth of yeast there is first a lag-phase which consists of a quiescent period of one to two hours, varying with the individual cells, then growth takes place at an unrestricted rate for a time and this in turn falls away. According to Slator (1918), measurements of the lag-phase in yeast show that old cells remain quiescent for a time when introduced into fresh medium. They then start growing rapidly at the normal unrestricted rate. On the other hand, little or no lag is shown in yeast cells grown from spores when once development has started. The growth of the yeast proceeds in alternate periods of rest and growth, and the observed lag is apparently little more than a prolonged period of rest.

If yeast be seeded into malt wort, the following stages of growth may be observed: (a) a lag-phase or period of quiescence; (b) a logarithmic phase or period of unrestricted growth; (c) retardation in growth due to carbon dioxide; (d) retardation due to failure of oxygen supply. It is this last factor which finally brings growth to a standstill. It is on the factors of seeding, aeration, temperature, etc., that the different stages of growth become prominent, overlap or disappear. If the seeding consist of actively growing yeast, the lag-phase disappears; if the seeding amount to a few million per cubic centimetre, retardation of growth at once sets in, and there is no logarithmic period; if the seeding

be very great, lag in growth and rapid accumulation of retarding influences may prevent growth entirely.

Gregory (1921) found that the increase in length, breadth and area of the leaves of *Cucumis sativus* show a grand period of growth, under normal conditions, in full sunlight. Three sets of experiments were undertaken; the seeds being sown in November, February and June, respectively. Each experiment lasted for

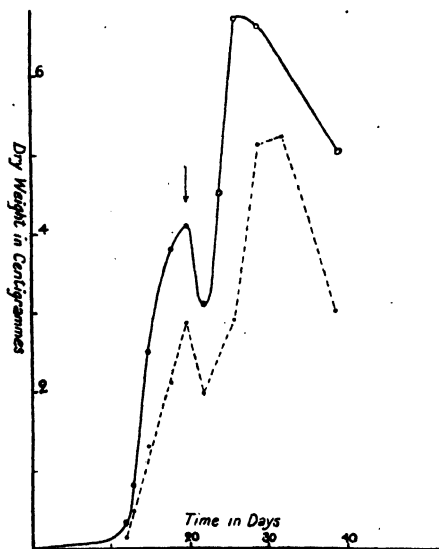


FIG. 46.—The dry-weight ratio for roots from cuttings of tomato (Princess of Wales). The arrow indicates the time of appearance of secondary roots. (After Priestley and Evershed, *Ann. Bot.*)

thirty days. The type of curve found for the growth of the leaves in November was of the typical S-shape, which is found for autocatalytic reactions; whereas, in March and June, the curves followed the exponential curves of the general type given by the equation:—

$$A = ae^{rt}$$

where  $A$  is the total area after a time  $t$  and  $a$  and  $r$  are constants. Such an expression may be written as:—

$$A = ax^t$$

where  $x = \log_e r$ , from which it will be seen that the growth followed a compound interest law. In December, however, the increase in leaf area followed a more complex law, which was expressed by the equation :—

$$A = at^r$$

where  $A$ ,  $e$ ,  $r$  and  $t$  had the same values as before, and the actual rate of increase at any time could be ascertained by the formula :—

$$\frac{dA}{dt} = \frac{rA}{t}$$

showing that the rate of increase was still proportional to the leaf area extant, but tended to fall away with time, owing to the presence of some detrimental factor. When the plants were grown in continuous artificial light, the rate of increase fell away with the first measurement in area. Gregory supposed that this detrimental factor might well be the high temperature which had to be maintained during the course of the experiments conducted under conditions of artificial light, and which led to an increase in the rate of respiration. Gregory has also found that the "average leaf area" was determined by the product and the intensity of the light.

Priestley and Evershed (1922) have quantitatively analysed the conditions necessary for the formation of roots from cuttings of *Tradescantia zebrina* and the tomato, based on calculations with wet and dry-weight material. The curves obtained were a sequence of S-shaped curves (Fig. 46). The time of transition from one curve to the next coincided with the time of appearance of a new crop of roots of a secondary order. In an interpretation of these results, Priestley and Pearsall (1922) looked upon the logarithmic phase of increase as the natural phase of increase, where increase in mass is an exponential function of the time; the phase of retardation, in which the growth rate is directly proportional to time, is said to be due to the operation of inhibiting factors, such as the accumulation of the end-products of metabolism which have been left over from the previous unrestricted growth.

Using the large mass of data gathered for the growth of maize

coefficient value lies between 2 and 3, so that the complete curve is not a Van't Hoff curve. The greatest growth occurs at  $30.3^{\circ}\text{C}$ . Leitch distinguished four main points from her results : (1) the minimum temperature, (2) the maximum temperature, (3) the optimum temperature, and (4) the maximum rate temperature. The minimum temperature is the lowest temperature at which growth takes place, the maximum temperature is the highest temperature at which the process will occur, while the optimum temperature is the highest temperature at which

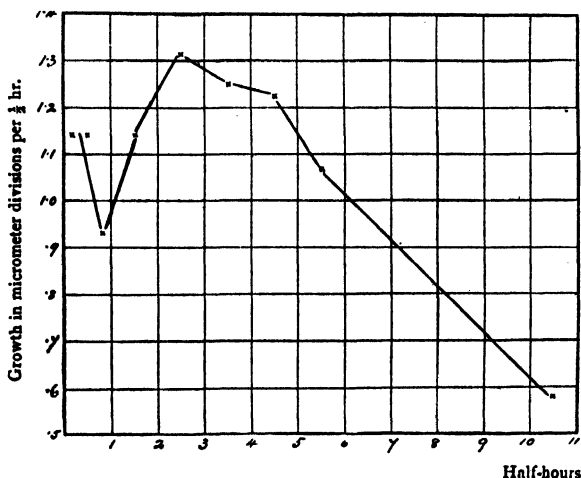


FIG. 48.—Graph of growth of pea seedling roots at  $35^{\circ}\text{C}$ . (After Leitch, *Ann. Bot.*)

no time-factor is involved, and the maximum rate temperature is that temperature at which the process attains its highest intensity.

The variations discovered by Leitch at higher temperatures may well be due to a number of factors, the powers of activity of which have been increased at unequal rates. The solubility of different food materials, the stability of various enzymes that are present in the cells, and the accumulation of the end-products of respiration are all affected in different ways by an increase in temperature.

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The growth of the total leaf area at higher temperatures conformed to the equation :—

$$\frac{1}{A} \cdot \frac{dA}{dt} = \frac{r}{t^n}$$

where  $A$  is the leaf area,  $t$  is the time that has elapsed from germination, and  $n$  a measure of the growth rate. The value of the exponent  $n$  increased with rising temperature : at  $76.8^\circ \text{F.}$ ,  $n = 0.517$  ; at  $84.4^\circ \text{F.}$ ,  $n = 0.613$  ; and at  $95^\circ \text{F.}$ ,  $n = 1$ . This indicates that with high temperatures, the falling off of relative leaf growth rate steadily increases.

The foliage leaves alone show growth curves which conform to a simple parabolic function :—

$$\log A = r \log t + \log A_0 ; \frac{1}{A} \cdot \frac{dA}{dt} = \frac{r}{t}$$

where  $A$  is the leaf area at a time  $t$ ,  $A_0$  is the leaf area at time unity, and  $r$  is the measure of the growth rate. At sub-optimal temperatures the value  $r$  remains constant, but falls rapidly at supra-optimal temperatures. The relative leaf growth rate is thus independent of temperature after the unfolding of the first foliage leaf.

According to Gregory, at sub-optimal temperatures relative leaf growth rate is independent of temperature, but is controlled by light intensity. Reduction of the light intensity or shortening the period of illumination leads to a smaller leaf area. The temperature affects leaf growth at sub-optimal temperatures by its action on the developmental rate in the apical bud, thus controlling the rate of unfolding of the first foliage leaf.

At supra-optimal temperatures a time-factor operates which tends to reduce relative leaf growth rate. This is due, in part, to a redistribution of material in the plant, more growth relatively by the stem, and, in part, to the direct effect of high temperature on the cell divisions in the leaf primordia, leading to a reduction in the number of cells produced before and after expansion.

At all temperatures employed in these experiments and with the light intensity used, there was a fall in the relative leaf growth rate with time. A second time-factor must therefore be considered as

operating due to the low light intensity. If light alone be operating detrimentally, this time-factor should disappear with increase in light intensity, and this was found to be the case.

### Electricity

The effect of electricity on the growth of plants has been investigated on and off for the last 180 years with remarkably poor results. The first pioneer in this branch of plant physiology appears to have been one Mr. Maimbray, of Edinburgh, who, according to Joseph Priestley in his "History and Present State of Electricity," published in 1776, electrified two myrtle trees "during the whole month of October, 1746, when they put forth small branches and blossomed sooner than other shrubs which had not been electrified." The Abbé Nollet, Court Physician to Louis XV., hearing of Maimbray's experiment, repeated it in France, and found that an electric discharge stimulated germination and growth of mustard.

Round about this time (1776) other views began to become prevalent. According to Koestlip and Ingen-Housz, electricity had a harmful effect on plant growth. In spite of the large amount of work that has been carried out on this subject with varying positive and negative results, none of the investigators concerned appears to have considered either the quantitative aspect of the matter or the plant. This quiet neglect of environmental and other factors concerned is not to be wondered at, since the workers in this field were either physicists, who were therefore unable to appreciate the biological side of the problem, or biologists whose knowledge of physics was of the scantiest nature.

Passing to more recent work, V. H. Blackman and Jørgensen (1917), working with an overhead electric discharge, have shown a greater appreciation of the complexity of the subject and the difficulties involved in an investigation of this kind. They were able to ascertain that oats treated with an overhead discharge show a marked superiority over untreated plants. The electrified plants were larger and of a deeper green than the

controls. The discharge in one set of experiments was continued for an average of five hours a day with the following results :—

	Total grain.	Total straw.	Percentage increase of grain.	Percentage increase of straw.
Electrified . . .	1309	2476	30	58
Control . . .	1008	1572		

The difficulty of confining the discharge to a special area was reduced by lowering the height of the wires. The amount of the discharge was 3 amperes at 50 volts.

In a second experiment two control plots were used, and the following values obtained :—

	June 18.	June 25.	July 3, 1916.
Electrified . . . . .	19	24	32 inches.
Control I. . . . .	14	19	21 „
Control II. . . . .	12	18	22 „

The electrified area gave a yield showing an increase of 20 bushels of grain over Control I., an increase of 40 per cent., while the increase in straw was 89 per cent. A marked after-effect was noticed in the electrified plot, for there was a considerable increase in the crop sown in the following year on the same ground.

According to V. H. Blackman and Legg (1924), who worked on maize and barley in pot culture and used a current of strength  $0.1 \times 10^{-10}$  amperes, there was a percentage increase in the growth of the maize of  $27 \pm 5.8$  and  $18.0 \pm 2.4$  in barley. It was also discovered that electrification of barley during the last month of the growing season was as effective as electrification during the whole season, and, further, that the discharge could be positive or negative in character. If currents of strengths  $1 \times 10^{-8}$  amperes or higher were used the effect was found to be injurious.

The effect of a glow discharge on the growth of the coleoptile of *Hordeum vulgare* has been investigated by V. H. Blackman, Legg

and Gregory (1923). A metallic point was charged to a high voltage and placed vertically over the coleoptile. The seedlings were grown in the dark in special culture tubes and a platinum wire was sealed into the glass so that the current actually passed

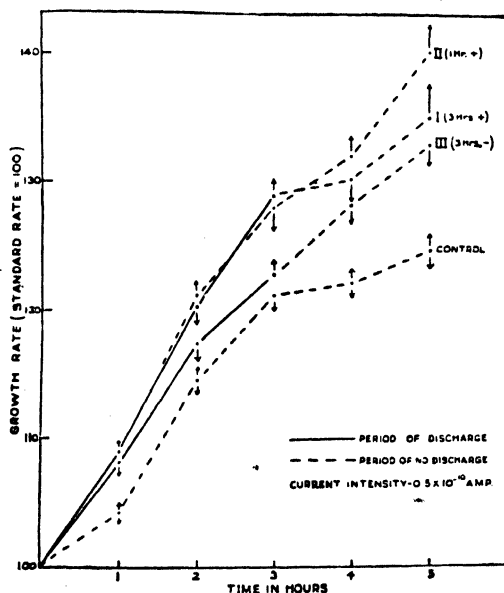


FIG. 40.—Graphs showing the mean hourly rates of growth of control coleoptiles and of coleoptiles exposed : (I.) to a *positive* discharge for the first three hours; (II.) to a *positive* discharge for the first hour; (III.) to a *negative* discharge for the first three hours. The growth rates are expressed as percentages of the "standard rate." The length of the arrows indicates the size of probable error of the various means. (After V. H. Blackman, Legg and Gregory, *Proc. Roy. Soc. Lond.*)

through the plant. The strength of the current used in the experiments was  $0.5 \times 10^{-10}$  amperes.

Since the individual coleoptiles showed a varying rate of growth, and, as the normal rate of growth increased during the course of the experiment, the results were expressed as "relative hourly rates," the rate of growth of the coleoptile for the hour immediately preceding the experimental period of five hours was

taken as the standard rate of growth for that coleoptile and expressed as 100. Plants were exposed (1) to a discharge of three hours with the point positively charged, (2) for one hour, and (3) treated to the discharge for three hours with the point negatively charged. It was found that during the discharge the current increased the rate of growth of the coleoptiles, and that the rate of growth was not only continued when the discharge was stopped, but grew even greater (Fig. 49). With the point negatively charged, this after-effect, although present, was not so great, and the rate of growth of the coleoptile tended to decrease during the discharge. This acceleration in the growth rate was entirely due to the current itself and not to the gaseous products of the discharge, for when a special platinum screen was inserted to cut off the current, no activation of growth was shown.

The growth of aberrant coleoptiles, however, mars the mean results obtained by these workers. In order to secure a uniform distribution of the effect of errors over the whole period of the experiment, Gregory and Batten (1926) held that in the estimation of the effect sought for, all measurements should have equal weight. They have achieved this by calculating from the hourly increments of growth, the "straight line of closest fit." A difficulty that was encountered in the course of the experiments was that the coleoptiles showed a well-marked rhythm in growth which had to be considered in making the calculations. The use of the formula :—

$$y - mx = c$$

where  $x$  represents the number of hours which have elapsed since the hour previous to the first hour of observation,  $c$  the growth during that hour, and  $m$  is the average acceleration of growth in an hour, considerably simplifies the matter. From an examination of the data two important points emerge : (a) that the slopes of the lines in corresponding series vary among themselves ; (b) even when the slopes are similar to the points of origin  $c$ , vary from experiment to experiment and are different in the control and electrified plants of a single series. By determining the correlation between the initial growth rate and the acceleration growth rate, a suitable correction may be

made for a variation in subsequent growth rate due to the initial rate. Using such corrected data the growth rates of the controls and stimulated coleoptiles appear as lines of varying slope. It was found in all cases (where corrected data were used) that all the lines showed positive results and demonstrated the markedly stimulating effect of the discharge.

Collins, Flint and McLane (1929) have investigated the effect of an overhead discharge on the growth of barley and maize. They found that a current of intensity  $10^{-9}$  amperes at night gave the most promising results with maize. The remainder of the experiments, however, did not give encouraging results, and in the majority of cases neither positive nor negative values for growth were obtained.

The precise reason why an electric current has a stimulating effect on plant growth is at present unknown. It was shown by Knight and Priestley (1914) that direct currents of  $10^{-6}$  and  $10^{-4}$  amperes do not increase or decrease the rate of respiration of germinating peas. An overhead discharge of  $3 \times 10^{-6}$  amperes also has no effect, while with higher currents there is an indefinite rise in the carbon dioxide output. This rise, however, was correlated with the rise in temperature due to the discharge. A further fact was discovered, that the gaseous products of the discharge have no effect on the germination of the peas, but are damaging to the seedlings. Further, the current apparently does not affect the photosynthetic rate. Marx (1929) has investigated the effect of electric currents on the assimilation rate of *Elodea canadensis* using Wilmot's bubble-counting technique. The products of electrolysis were not allowed to reach the plant and the strength of the current<sup>1</sup> was  $1.15 \times 6 \times 10^{-7}$  milliamperes per square centimetre. With such a current there was no increase or decrease in the assimilation rate. A current of 1 milliampere per square centimetre had a decidedly depressing effect. It is thus evident that the stimulating effect of electric discharge must be looked for elsewhere than on either photosynthesis or respiration.

<sup>1</sup> The current density was calculated in terms of area of cross-section of the mass of water between the electrodes which was about 1720 cm.<sup>2</sup>

### Climatic Factors

The effect of such environmental factors as light, humidity and temperature on the growth of plants living under field conditions belongs more especially to a discussion on ecology, but certain limited aspects of the matter must be considered here. One or more of the above factors have already been considered in their individual action on the living plant; their mass effect has now to be discussed.

Hildebrandt (1917) has suggested that the leaflet measurements of the soy-bean might be used as a standard to measure the "climatic effectiveness" in the United States. McLean (1917) has employed the method to investigate the climatic action on the soy-bean in various parts of the U.S.A. The following measurements were made after two weeks and during the first month from sowing, at Oaklands (in the mountains of Maryland), and at Easton (on the eastern side of Chesapeake Bay): stem height, average number of leaves per plant, average length and width of mature leaves and the leaf-product, which was obtained by multiplying length by width of each leaf, the average leaf area, and the dry-weight of the tops of the plants.

It was found that the rates of growth in terms of leaf surface and in terms of dry-weight varied in a similar manner with the same kind of variations in external conditions; whereas, the growth rates measured in terms of stem elongation, varied in another way with similar external conditions. The seasonal marches of the growth rates of the Oaklands plants were found to be considerably different from those prevailing at Easton. It would seem that temperature was clearly the limiting factor for growth during the first fortnight. During the second fourteen days, however, with similar external conditions prevailing, the moisture relation (rainfall/evaporation ratio) appeared to be the limiting factor for growth, this being especially the case when the temperature was high. Thus it can come about with two plants in different phases of their growth and development, exposed to the same fluctuations of external conditions, that the limiting factor for one case may be very different to the limiting factor for

the other. McLean pointed out that this may well be due to the difference between the internal conditions of the plant at different phases of its development.

Hildebrandt (1921) studied the effects of the climatic complexes on plant growth in nine different stations in Maryland for the summer of 1914. The effect of each complex was automatically integrated for soy-bean plants for a period of four weeks from sowing, new seeds being sown every two weeks. Measurements of stem elongation, leaf area, "leaf-product" and dry-weight were taken, and the environmental factors studied were air-temperature, evaporating power of the air, and the intensity and duration of sunlight. Indices of the total seasonal climatic efficiency derived by multiplying the seasonal average growth rate per day by the normal length in days of the growing season for the station in question were found to have the following values :—

Oaklands . . . . .	9,009
Chewsville . . . . .	12,480
College . . . . .	16,867
Easton . . . . .	17,688
Princess Anne . . . . .	19,005
Coleman . . . . .	21,115
Darlington . . . . .	23,688
Baltimore . . . . .	25,422

The previous history of a plant has an important bearing on its subsequent behaviour. From a large number of experiments Balls (1918) concluded that the different behaviour of the plants, indicated by the crop of cotton, was the result of known environmental factors, provided always that true regard was paid to the distinction in time between the incidence of the conditioning factor and its manifestation in the crop. Thus the daily fluctuations in the flowering curve were determined and controlled by weather conditions which were in force a month before the flowers opened. The importance of this principle of predetermination is very great, and has not sufficiently been realised in previous work. Brenchley (1920) studied the effect of environmental factors on the growth rate of garden peas. The plants were grown in water

by Kreusler and his co-workers, G. E. Briggs, Kidd and West (1920) have analysed the growth of this plant in terms of dry-weight, leaf area, and time, and have also employed the measure "relative growth rate" which they defined as the weekly percentage increase in dry-weight plotted against time and the "leaf area ratio" which was the leaf area increase in square centimetres plotted against time.

It was found that the growth rate of maize varied at different times of its life in a perfectly definite manner, and it was discovered that in the first stage there was an actual decrease in weight, evidently to be correlated with losses due to respiration. Then followed a phase of rapid increase rising to a maximum which passed into a steady and continuous fall. Subsidiary maxima made their appearance in the descending part of the curve which coincided with the appearance of the male and female inflorescences. It is possible that these secondary maxima which made their appearance were due to strong secondary reactions, for there was a marked rise in respiration at such a time. Of the various environmental factors, temperature is said to have more bearing than light.

In a second paper of this series, Briggs, Kidd and West (1920) have expressed the rate of growth per unit of leaf area instead of unit dry-weight, and have made use of a third relation "unit leaf rate," which is defined as the "weekly rate of increase of dry-weight in milligrammes per square centimetre." They found that the unit leaf rate did not undergo a perfectly definite type of variation as did the relative growth rate, but fluctuated about a mean value. It was this unit leaf rate which was so closely correlated with temperature rather than with any other external factor.

The growth curves of the cotton plant have been analysed for the conditions prevailing in India by Inamdar, Singh and Pande (1925). The growth curve showed a maximum increase which was reached sooner or later, depending on the vegetative phase. The shorter the vegetative phase, the earlier was the maximum reached. No grand period of growth was discovered. Initial comparison of the growth rate curves with the leaf area ratio and

culture and the rate of growth was expressed in terms of dry-weight. During the early seedling stage the rate of growth was associated with relatively warm days and nights, and bright sunshine had little significance. During the later periods of growth, increase was strongly associated with sunshine and warm days, but not significantly with night temperatures. These results were to be expected. In the early seedling stage the photosynthetic mechanism had not yet reached its full development, and therefore the young seedling could not make full use of the sunshine, while, in the later stages of growth, the perfection of the assimilation apparatus at once demonstrated the marked significance of sunshine.

Mason (1922) has made a survey of the various external and internal factors affecting the shedding of the bolls and flower-heads of cotton in St. Vincent. He was able to show that the susceptibility to shedding became especially pronounced after growth-cessation in the main axis. This cessation of growth and increased susceptibility to shedding was found to be due to the deflection of the supply of elaborated food material from the apical portion of the plant to the fruit developing on the basal fruiting branches.

Overcast, humid days retarded the growth of the main axis ; and low rates of evaporation, little direct solar radiation and periods of daytime rain were forerunners of increased rates of shedding. Mason attributed the retardation of the growth rate and increased rates of shedding to the check in the photosynthetic activity of the leaves, and drew the general conclusion that the proportion of shedding over any given period of time was the resultant of two opposing factors : (1) the rate at which food was synthesised by the plant ; and (2) the rate at which it was utilised in the maturation of the fruit, and any check to (1) increased the rate of shedding.

Gregory (1926) has published a communication of considerable importance to the elucidation of climatic factors on the growth of barley. The barley was grown in pot culture and the water-content of the soil was maintained at 15 per cent., the optimum for the type of soil employed in the experiments. The seeds were graded and six sown in each pot. After germination three of the

seedlings were removed and the three most uniform seedlings left. Two types of manurial treatment were used : (a) 0.5 gm. sodium nitrate, 1 gm. superphosphate, 0.25 gm. potassium sulphate per plant ; and (b) 1 gm. sodium nitrate, 1 gm. potassium nitrate per plant. The assimilation rate in the two series proved to be the same. Three measurements of growth were employed : (1) net assimilation rate (dry-weight increase per unit leaf area per unit time) ; (2) relative growth rate of leaf surface (rate of growth per unit weight) ; and (3) relative rate of increase in dry-weight—the efficiency index (see p. 250). Measurements were also made of the environmental factors concerned : (a) maximum day temperature, (b) average day temperature, (c) minimum night temperature, (d) average night temperature, (e) total radiation in calories per square centimetre per week ; (f) hours bright sunshine, and (g) evaporating power of the air.

Dividing the growth cycle into two parts, Gregory found that the first part of the growth cycle was independent of the time and quantity of nitrogen added to the manure. There was a positive correlation of the assimilation rate with the day temperature and radiation, while there was no such correlation with the night temperature. In the absence of bright sunshine, in so far as the temperature did not fall, the high relative leaf growth rate led to a large leaf surface and so compensated for low net assimilation or *vice versa*. This compensating effect, however, was partially masked by high nitrification rate in the soil which was associated with high soil temperature, and hence with total radiation, which in any case brought about an increase in leaf area. Secondly, relative leaf growth rate was largely independent of external factors since the leaf is the metabolic factory of the plant, and is independent, relatively speaking, of external factors.

The influence of climatic factors on the growth of oats has been studied by Alun Roberts (1928), and Tincker and M. G. Jones (1931). The oat plant is essentially a lover of cool conditions in its later life period. A warm, dry seed bed is essential for high yields, while a cold, wet seed bed gives grain yields below the normal. According to Roberts, who has statistically examined the data obtained for oat trials between the years 1908 to 1926 at Aber,

Bangor, Wales, it is the weather conditions prevailing at the time of emergence of the panicles which is the critical factor governing ultimate yield. Abundance of moisture, together with the absence of excessive heat from the flowering period to the time of ripening, favours high yields. On the other hand, the presence of high temperatures and heavy rainfall in the later stages of the development of the grain may prove particularly damaging to the resultant yields. Tincker and Jones have found that either unit leaf area or net assimilation rate in the oat (see above) are most closely correlated with both temperature conditions and rainfall. Furthermore, the relative growth rate or Blackman's "efficiency index" (see section on Growth Curves) showed no statistically significant correlation with the climatic factors examined. A negative correlation was discovered between increase in leaf area and temperature, a result to be expected, as the oat is a cool-loving cereal.

Appleman and Eaton (1921) found that temperature was the controlling factor of the rate of ripening of sweet corn under field conditions. For a wide range of temperature the rate of ripening strictly follows the Van't Hoff law.

### **The Frost Resistance of Plants**

The influence of intense cold on plants is of economic importance in Europe and the American continent, and a number of investigations have been made on this subject to determine the precise action of extremely low temperatures on plant cells.

The effect of frost and low temperatures has been investigated by R. B. Harvey (1918, 1919). Using various succulent plants, Harvey found that one of the first effects of frost injury is the appearance of injected areas on the leaf, due to the withdrawal of water from the cells and the subsequent displacement of air in the inter-cellular spaces by this water. In some plants the frozen cells may become stimulated into abnormal growth, and tumour-like outgrowths are produced very similar in appearance to those produced by parasitic fungi and bacteria. He compared the resistance to low temperatures and freezing of untreated and

"hardened" plants of tomato and cabbage by exposure for a week or more to temperatures slightly above the freezing-point, and found in the cabbage that plants which had been kept for five days at 3° C. withstood exposure for half an hour to - 3° C., whereas the control plants were killed. Harvey considered that the chief effect of "hardening" is due to a change in the proteins of the protoplasm which prevents their precipitation as a result of the physical changes following on "hardening." Changes in the carbohydrate values are slight, whereas the amino-acid content increases. Any changes in the sugar content causing an increased depression of the freezing-point, or the nature of the epidermal cells, which may affect undercooling of the tissues by prevention of inoculation from ice formed on the surface, are relatively unimportant. Harvey (1922) has obtained very similar results for lettuce.

Tuttle (1919) and Lewis and Tuttle (1920), in an investigation of induced changes in the reserve material of evergreen herbaceous plants, measured periodically from autumn to summer the osmotic pressures, electrical conductivities, proportion of electrolytes, as well as the amounts of glucose, maltose and sucrose in the leaves of *Picea excelsa*, *Linnaea borealis* and the cortical tissues of *Populus tremuloides*. No definite correlation could be found between these values and either daily or weekly variations of air-temperature. The sugars, on the other hand, showed a marked concentration during the winter and a progressive decrease from the maximum amount in the winter to the summer period.

A microscopic examination of the mesophyll structure of leaves of *Picea excelsa*, which had been subjected to a severe winter in North-West Canada, showed that the identity of the chloroplasts which appeared bright green in colour closely associated with the nucleus, was completely lost. During the autumn the whole of the starch content had disappeared and appeared to have been replaced by oil. Ice, according to these investigators, only makes its appearance in the living cells of *Pyrola* at temperatures below - 31° C.

It must also be borne in mind in this connection that trees and shoots in cold climates become dormant at the close of the growing

season without exposure to cold weather. Coville (1920) therefore considered that the generally accepted view that the dormant conditions exhibited by plants in winter are due to low temperature is not necessarily correct. He brought forward the theory that the effect of low temperatures in dormant plants is closely connected with the transformation of starch into sugar, a change brought about by the weakening of the cell membranes which become permeable to amylolytic enzymes, and these then act upon the starch grains stored in the cells. He also gave experimental data in support of this hypothesis to show that growth may be stimulated by any process which will cause local injury to the tissues.

Johnston (1919, 1923) suggested that the ratio of water-content to dry-weight might serve as a possible index for the measurement of the winter hardiness of fruit buds. According to this same investigator (1922) there is a decrease in the hardiness of fruit buds with the approach of spring. Data are presented to show that wet buds freeze at a considerably higher temperature than dry buds. It follows from this result that a cold spell following rain is especially dangerous to fruit trees. According to West and Edlefsen (1921), the buds of peach, cherry and apricot, show different degrees of hardiness to frost at different stages in their development, and these differences are considered to be due to changes in the quality and quantity of the cell sap.

R. Newton (1922) in an investigation of the winter hardiness of wheat found that all the varieties used in his experiments increased in amino-nitrogen and water-soluble nitrogen during hardening. The hardiest variety had the largest quantity of water-soluble nitrogen, but the relation was not constant throughout the series. The colloidal complex of fully hardened tissue was very resistant to freezing and could not be broken down by a freezing mixture with a theoretical temperature of  $-59.9^{\circ}\text{C}$ . Newton (1924) also ascertained that the imbibition pressure of fresh leaves in the winter-hardened condition as determined by the pressure required to express the tissue fluids was in most cases directly related to hardiness. In one case an imbibition pressure of 600 atmospheres was recorded. With unhardened leaves no such relation could be

discovered. The imbibition pressure of hardened leaves appeared to depend on the physical state of the cell colloids characteristic of living tissues, since this property was lost when the tissues were killed. It was found that the moisture-content of hardened tissues tended to be inversely proportional to hardness. The hydrophylic colloids contained in the press juice were found to be directly proportional to hardness. There appeared to be a certain amount of evidence that the ratio of amino-nitrogen to total nitrogen increased in all varieties in the late fall, indicating an association of protein-splitting with the later stages of the hardening process.

It was originally suggested in 1920 by MacDougal that pentosans were of the greatest importance in relation to the frost resistance of plants. This view has been extended by Hooker (1920) who considered that the pentosans, or rather some specific pentosan, functions in the plant tissue by holding water which is in the nature of adsorbed or colloidal water, and that this type actually does not freeze when the plant is subjected to ordinary winter conditions. The greater water-content found in tender tissue as compared with more hardy tissue is therefore due to an excess of free water. On the other hand, although hardy tissues contain less free water, they contain more adsorbed or colloidal water. It was found that the shoots of hardy varieties of apple, such as Wealthy and Yellow Transparent, always contained a higher pentosan content than the tenderer varieties, such as Missouri Pippin. In the majority of cases the base of the shoot contained more pentosan than the tip. Similar results have been obtained by Rosa (1920) for vegetables. Plants hardened by exposure to low temperatures or by withholding moisture showed a higher pentosan content than non-hardened plants, and the pentosan content increased with the hardening process. Rosa advanced a theory similar to that of Hooker to account for winter hardness.

R. Newton and Brown (1926) could find no support for the suggestion that the pentosan content is correlated with hardness in wheat. The bulk of the colloids in the cell contents consisted of proteins; 90 per cent. of the total protein of the plant being

contained in the extracted fluids. The pentosans were almost entirely confined to the structural regions of the plant. Concentration of sugars in the plant increased most in the hardest varieties, thus giving them the greatest resistance to frost denaturation of proteins. It would therefore appear that the pentosans play little if any part in frost resistance. Similarly, Doyle and Clinch (1926) found that there is no apparent connection between cold resistance and pentosan content of the leaves of conifers.

### **The Carbohydrate/Nitrogen Ratio**

Kraus and Kraybill (1918), in an elaborate biochemical investigation concerned with the nitrate and carbohydrate content of the tomato and the responses correlated with their presence, were able to recognise four main conditions :—

I. Though there be present an abundance of moisture and mineral nutrients, including nitrates, yet without available carbohydrate supply, vegetative growth is weakened and the plants are non-fruitful.

II. An abundance of moisture and mineral nutrients, especially nitrates, coupled with available carbohydrate supply, makes for increased vegetation, barrenness and sterility.

III. A relative decrease of nitrates in proportion to the carbohydrate makes for an accumulation of the latter and also for fruitfulness, fertility and lessened vegetation.

IV. A further reduction of nitrates without inhibiting a possible increase of carbohydrates makes for a suppression both of vegetation and fruitfulness.

According to Kraus and Kraybill, "fruitfulness is associated neither with the highest nitrates nor with the highest carbohydrates, but with a condition of balance between them."

It was hoped that this work would give an impetus to the discovery of the underlying physiological reasons for such empirical horticultural practices as pruning and manuring. These investigators recognised the fact that fruit production is apparently a specialised vegetative function, usually more or less closely

associated with gametic reproduction, and that the conditions for the initiation of flower primordia and even blooming are different for those accompanying fruit setting.

Since this original publication, a wealth of papers has been produced dealing with the carbohydrate/nitrogen ratio in different plants on the lines of Kraus and Kraybill and the subject has developed into quite a "vogue" among plant physiologists. Attempts have been made on all hands to explain nearly every manifestation of the plant with this ratio, although it is probable that Kraus and Kraybill themselves never meant to make the elaborate claims that later workers have superimposed on their paper.

As Work (1924) has pointed out: "When two factors play as complex and various parts in plant metabolism as do nitrogen and carbohydrates, each being present in more than one form and each being assigned a multiple rôle, it is hardly to be expected that their relation to each other may be expressed by a simple mathematical ratio. This would imply that the two are interdependent variables, and that together they constitute a factor which conditions the activity of the plant—in this case, vegetative and reproductive activity."

Almost immediately following the work of Kraus and Kraybill, Woo (1919) published the results of his investigations on *Ameranthus retroflexus*. This plant has apparently an almost abnormal capacity for the absorption and retention of nitrates. Large quantities of nitrates may be present and yet the plant may not be "forced out of reproduction," although the carbohydrate/nitrogen ratio is low. It would therefore appear from the results of this investigation that the ratio is different in different plants producing the same range of effects. This is an obvious conclusion, and Gurjar (1920) found that the ratio for tomato may vary between 2 and 19, but fruiting always occurred when the ratio was between 4 and 6.

The question of the application of the results of Kraus and Kraybill to the apple tree is beset with difficulties. In the apple, the fruiting spurs are all at the same stage of development at the same time, and it would thus appear that a more reliable indication

of the relation between chemical composition and fruiting may be obtained if the investigations are confined to this part of the plant. Hooker (1920) has found that by correlating the carbohydrate and nitrogen content with the stage of development of the apple spur at the onset of the non-blooming year, there was low nitrogen and high carbohydrate content present, and that throughout the winter loss of both occurred up to the formation of the terminal buds. In the spring and summer, photosynthesis built up carbohydrate reserves, and the nitrogen still remained low. These conditions are considered to ensure fruit-bud formation. With the formation of the buds, the C/N ratio (carbohydrate/nitrogen ratio) changed rapidly within the spur, and the nitrogen accumulated to its highest concentration. Since the low C/N ratio brought about much vegetative growth during the summer and previous spring, the increased ratio now brought about flowering and fruit production. Hooker (1920) attempted to apply his results to orchards by testing the effects of nitrogenous manuring at different periods of the year. The age of the tree is considered to be an important factor in this connection and the time of the year at which the manure is applied is also important.

E. M. Harvey and Murneek (1921) have shown that spurs defoliated in June have a lessened C/N ratio due to increased nitrogen and decreased carbohydrate, and as a result of this defoliation there was a decrease in flowering. E. M. Harvey (1923) has studied the rôle of carbohydrate and nitrogen upon the growth rate after defoliation and ringing at different periods of the year. He found that defoliation retarded growth considerably if it were effected during the period of maximum growth rate. On the other hand, defoliation earlier in the season showed a smaller retarding effect, though, if the defoliation were performed late in the season, there was very little retardation of growth, and it might even be accelerated. The result of ringing shoots was in general opposite to defoliation.

R. H. Roberts (1921) found evidence that apple trees may store a reserve of nitrogen in one season which may be utilised in a later season when the external supply is low. He found that a high

C/N ratio in the buds, *i.e.*, buds with a low nitrogen content, leads to little growth and the complete suppression of fruit-bud formation. However, a low ratio leads to much vegetative growth and very few fruit buds. An intermediate value of the ratio leads to vigorous vegetation and abundant fruit buds.

Gardner (1923) has attempted to apply the results obtained for apple spurs to strawberries. The yield of fruit is found to depend upon the nutritive conditions prevailing in the autumn at the time of formation of the fruit buds. The maximum number of flower-head clusters is obtained when the conditions during the previous autumn are such as to provide a high carbohydrate content.

Hicks (1928) has considered the question of the C/N ratio in its relation to the growth of wheat. Only total carbon and total nitrogen were determined, and no attempt was made to distinguish between different carbohydrates. In the three strains of wheat used in these experiments: Marquis, Nevin Bearded and English Spring wheat, it was found that the embryos tend to reproduce similar C/N ratios, irrespective of the amounts of carbon and nitrogen in them. Early stages of germination were characterised by a low C/N ratio. Vegetative activity reduced the nitrogen percentage steadily and the carbon rose to a maximum at about half-way through the life-cycle. The percentage of carbon fell considerably before blooming, and when a sufficiently high C/N ratio arose, flowering occurred.

This investigation is somewhat in the nature of the contemplation of the obvious, and the results could have been predicted without the unnecessary toil of microanalysis which the author carried out with such laborious care. No possible significance can be placed on results which include the total carbon of the plant, because the continued production of dead tissue, such as xylem and sclerenchyma, will, of necessity, play an important rôle in the estimations and significance of the final results. More especially does this become apparent when the fact is considered that the nitrogen, in contrast to the carbon, is practically entirely used in active metabolism.

The significance of the C/N ratio in connection with photo-

leaf weight ratio showed no agreement between them. This was possibly due to the fact that the leaves had not yet reached their maximum assimilating capacity. There was an active phase of growth when the growth rate curve ran parallel to the curve of either the leaf weight ratio or the leaf area ratio curves. It was further ascertained that there was a greater decrease in the growth rate curve in the later stages of the life-cycle of the plant than could be accounted for in the percentage leaf weight ratio or leaf area ratio.

V. H. Blackman (1919) considered that the growth of an annual plant follows a compound interest law. He pointed out that in many natural phenomena, processes are to be found in which the rate of change is proportional to the quantity itself. Money placed at compound interest accumulates in this way, and the rate at which a body cools follows a compound interest law. Blackman went on to show that assimilation and growth are closely correlated: "If the rate of assimilation per unit area of leaf surface and the rate of respiration remain constant, and the size of the leaf system bears a constant relation to the dry-weight of the whole plant, then the rate of production of new material, as measured by the dry-weight, will be proportional to the size of the plant, *i.e.*, the plant in its increase of dry-weight will follow the compound interest law."

In any annual plant the ultimate dry-weight will depend (1) on the weight of the seed; (2) on the rate at which the material present is employed to produce new material, *i.e.*, percentage increase in dry-weight per day or week or other period; and (3) the time during which the plant increases in weight.

Gressler (1907), working with *Helianthus annuus*, treated the matter as though it were a discontinuous geometric series. Blackman held it to be continuous, a very much more probable assumption, and substituted the equation:—

$$W_1 = W_0 e^{rt}$$

to express the growth of an annual plant rather than Gressler's equation:—

$$W_1 = W_0(1 + r)^t$$

periodism will be considered in the next chapter and will not be further discussed here.

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## CHAPTER VIII

### GROWTH—*continued*

### LIGHT AND GROWTH

*Etiolation—Photoperiodism—Effect of Different Parts of Spectrum on Growth—Polarised Light and Growth—Light and Reproduction.*

#### **Etiolation**

IN the vast majority of cases light is a large factor in the growth of plants. The effect of light is fundamentally different from that of heat. A number of fungi, for example, can pass through the whole of their life-cycle in darkness, while in the greater number of land plants the root system is intolerant of light. In general terms it can be said that light possesses a formative influence, and is also indirectly important, since it provides the necessary energy for photosynthesis.

Light has a strong formative influence on plant organs, such as stem and leaves, which are markedly altered morphologically by prolonged exposure to darkness. Apart from alterations in the colour of the green pigments of the stem and leaves, plants exhibit other characteristics when exposed to darkness; peculiarities which are summarised in the term *etiolation*. Etiolation phenomena are best studied in plants with abundant supplies of reserve food, such as the dahlia or potato, since darkness deprives a plant of its assimilating powers. The general characteristics of etiolated plants are prolonged internodes and a suppression of the leaf lamina which becomes scale-like in shape. The xylem does not reach the same development as that of the normal plant grown in the light, and the etiolated shoot is soft, sappy and weak.

According to Priestley and Ewing (1923), the stems of *Vicia*

*Faba*, *Solanum tuberosum*, and *Pisum sativum* develop a true primary endodermis with Casparian strip when grown in complete darkness, in place of a starch sheath. This endodermis is considered to restrict the supply of nutrient sap necessary for the growth, to tissues within the endodermal cylinder and the apex. The development of the endodermis in the dark is said to account for the excessive elongation of the stem, as only the cells at the base of the meristematic tissues which cap the end of the endodermal cylinder receive adequate supplies of food. Growth of this meristematic tissue adds to the length of the stem, and growth in length is favoured at the expense of the normal lateral growth of leaf and cortex ; thus, for example, the angles of the stem of *Vicia Faba* opposite the main vascular bundles fail to develop, and the stem remains rounded in cross-section. Occasionally it was found in *P. sativum* and *V. Faba* that on prolonged exposure to darkness, the Casparian strip may fail to develop though growth continues, and in such circumstances there is a greater development of lateral leaf extensions. In *Phaseolus multiflorus* no endodermis develops in the dark, and therefore the stem remains rounded in outline. Priestley (1926) claimed that in *V. Faba* a constant feature of the etiolated shoot is that the cortical bundles seem to divide relatively much earlier than in the non-etiolated plant. In the etiolated shoot the starch sheath is replaced by a primary endodermis. A striking stain which shows up this endodermis is Nile Blue. From the reactions of the layer to osmic acid, the staining with Nile Blue seems to be due to the presence of fatty substances. The cells below the meristematic region are very fully packed with starch. Priestley concluded that the main morphological and structural features associated with etiolation are determined by a redistribution of meristematic growth at the shoot apex, following on the greater difficulty experienced by the meristem in drawing nourishment from the vascular supply, because when grown in the dark, the walls between the vascular strands and meristem are rendered relatively impermeable by the retention within them of protein and fatty material that form the surface of the protoplast.

It would appear to place a heavy burden on a single layer of

cells to claim that great morphological changes in the plant body are brought about by their presence, and it is difficult to conceive why the migration of fats should lead to these alterations in shape. The plant is a complex metabolic machine, and, as Gregory (1928) has pointed out, the action of light "is not inconsistent with a hypothesis of a 'master' photochemical reaction independent of carbon assimilation leading to the formation of a substance directly involved in leaf expansion." There is a minimum light intensity below which growth cannot proceed, and unless this level of light intensity is maintained, a time-factor makes its appearance, leading to continuous reduction in relative leaf growth rate. This reduction of growth rate may well be due to a fall in the growth rate of the roots. In barley, certainly, root growth and leaf growth are closely correlated. This close correlation may be due to the fact that the leaf supplies carbohydrate which is important for the growth of the roots, and the roots supply nitrate which is necessary for the growth of the leaves. The action of hormones in plant physiology is only now beginning to be realised, and no doubt further investigations will lead to considerable extensions of our knowledge of the part they play in plant metabolism.

Short exposures to light have a marked effect on the development of the leaf lamina in etiolated plants. Trumpf (1924) exposed plants of *Phaseolus multiflorus* for varying periods of time, such as thirty minutes, two hours, four hours and twelve hours, to artificial light, and found that even the shortest exposure to light produced profound morphological changes. The internodes were much shorter and the laminae broader. In a second series of experiments the plants were illuminated for one, five, ten and thirty minutes, and closely resembled plants exposed to a longer period in light, with the exception that plants having the longest daily exposure (ten to thirty minutes), although having well-developed laminae, showed no development of chlorophyll. Thus, in changes of form, the quantity of light is the factor concerned, and its action is not produced indirectly on the assimilating system. Trumpf also ascertained that in light of different wave-length, the blue rays and, to a certain extent, the red rays

favoured elongation of the stem, while the blue light markedly induced lamina formation. Priestley (1925) has confirmed these observations (Fig. 50), and also found that completely etiolated



FIG. 50.—Effect of brief light exposure on the broad bean (*Vicia Faba*). Reading from left to right, 4 minutes daily ; 2 minutes daily ; 1 minute daily ; continuous darkness. (After Priestley, *New Phyt.*)

plants show no signs of lateral leaf development and always retain their plumular hook.

### Photoperiodism

The effect of the duration of light on growth, the so-called *photoperiodism*, has been studied with great intensity during the last few years, and a large literature now surrounds this aspect of the subject. Only the more important publications will be considered here, as the mass of facts now available are too bulky to review in anything more than brief detail.

Garner and Allard (1920), working with the soy-bean, tobacco and other plants, found that vegetative growth in these plants was proportional to the duration of exposure to daylight. Short exposures led to the production of small slender plants with a slower growth

rate than plants exposed to light for a longer period. They also showed that light is an important factor in the inception of the reproductive phase. By suitable modifications of exposure they found that annuals could be converted into biennials, and many biennials could be made to flower in a few months. In the absence of a particular day-length, vegetative growth can be made to continue for an indefinite period. Garner and Allard found that the majority of species they investigated could be placed in one of two groups in regard to the response they made in reproductive activity to varying durations of light. To one group they gave the term "short day" plants, and to the other "long day" plants. In the "short day" group the flowering stage was accelerated by a relatively short daily exposure to light, whereas the reproductive phase was delayed or inhibited by a long exposure to light. On the other hand, in the "long day" group a much smaller group than the "short day" one, prolonged duration of light hastened the onset of flowering, whereas a short exposure retarded it.

In a further series of investigations Garner and Allard (1928) were able to show that with sufficient departure from the optimum amount of light, growth of the primary axis may be completely suppressed and aerial growth practically confined to leaf development; a type of development typified in rosette plants. Another manifestation of the sub-optimal action of light is the creeping or prostrate habit. } Tuberisation is also said to be a feature of photoperiodism. It was shown, as far back as 1887 by Vöchting, that tuber-formation in the potato is favoured by darkness; even darkening of the primary shoot or a branch will convert the growing point into a tuber. Garner and Allard (*ibid.*) found that with exposure to a ten-hour day there is a permanent failure to form tubers in the onion, while with five hours of daylight the plants were of a weak green colour and showed no signs of forming bulbs, but the flower heads appeared, although they never opened. Under increased illumination, potatoes failed to form tubers, and here again the flowers were unable to open.

Reduction of the light period below the optimum for stem elongation appears to lead to sexual reproduction, while decrease

of the optimum for sexual reproduction leads to intense tuberisation ; a stage marking the final reduction of stem elongation.

It must be borne in mind that in the winter months the relation between the length of daylight and the temperature becomes especially important. On account of the shortness of the winter day, increase of the plant through photosynthesis is reduced to a minimum and growth conditions are otherwise unfavourable, so



FIG. 51.—Effect of different periods of light daylight on *Dactylis glomerata* (Cocksfoot). Left to right : 12 hours, 9 hours, 6 hours and control. (After Tincker, *Ann. Bot.*)

that the advent of high temperatures is likely to establish an unsuitable ratio between income and outgo, and this may easily prove to be disastrous for the plant.

Garner, Bacon and Allard (1924) found that in addition to the action of the light on the fundamental process of assimilation, the duration of the light may also control other parallel processes important to plant growth. In some way the action of light produces a profound influence on the acidity relations in plants. In the case of "short day" plants, *i.e.*, those plants which are able

to flower with a minimum amount of daylight, upward elongation of the stem is a characteristic response to a relatively long daily illumination period and is associated with an increase in the active acidity of the plant, particularly near the growing point. In "long day" plants exposed to relatively "short day" periods, stem elongation is prohibited and conditions of acidity remain at a low level.

In their main aspects, the results of Garner and Allard have been confirmed by Tincker (1925) (Fig. 51).

More recently Garner and Allard (1925), using ventilated light-proof boxes having three sides removable, were able to expose different portions of the primary stem of *Cosmos sulphineus* to daily periods of illumination, and in certain cases to periods of darkness. When the upper portion of the stem was exposed to the full length of a summer day and the lower portion to ten hours of light only, the latter soon flowered, while the former remained vegetative. Similarly, exposure of the central part of the axis to a long day, while the upper and lower portions received only short exposures, led to the production of vegetative development of the former, while the latter (*i.e.*, the upper and lower portions of the stem) flowered promptly. By reversing this treatment the central portion of the axis was made to flower, while the upper and lower portions remained vegetative. Exposure of the upper region of the stem to darkness for three to five weeks, while the lower region received "short day" exposure, resulted in the development of flower-heads in the upper darkened region. When, however, the lower region of the axis was exposed to the action of a long summer day, no flower-heads appeared in the upper darkened region.

Adams (1923, 1924) grew buckwheat, hemp and other plants in light and darkness. The greatest amount of growth was found in the latter at first, but those exposed to light showed the greatest growth in the end. The whole matter appears to be connected with the reserve food material present for the formation of new tissues. If two plants have the same quantity of reserve food, the one grown in diminishing light will show the greatest growth (growth here being elongation in length of stem and not increase

in dry-weight) while the stock of food lasts. In the case of the tomato, soy-bean, hemp and buckwheat, there appears to be an upper limit of light beyond which the plant can make no further growth. In almost all cases plants exposed to the longest day gave (a) the greatest average dry-weight, (b) the greatest average height, and (c) the earliest flowers.

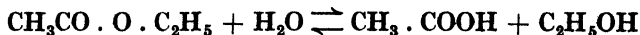
Temperature and humidity also play a part in influencing the response of the plant to relative day length. Gilbert (1926a), working with cotton and soy-bean, found that these plants exhibit definite reactions to higher and lower temperature conditions. There is a perfectly definite retardation of flowering with low temperatures and high humidity. *Cosmos*, on the other hand, flowered much earlier and more abundantly with low temperature and high humidity, while *Salvia* and buckwheat showed no response to either high humidity or low temperature. Experiments on the effect of relative day length and temperature show, according to Gilbert (1926b), that temperature is a determining factor in influencing the time of flower primordia formation in *Xanthium pennsylvanicum*. Temperature and relative day length are particularly closely correlated in this connection. The interrelation of day length and temperature are said to be as follows: With high temperatures, short-day plants (plants exposed to daylight for ten hours) give indications of flowering from twelve to fifteen days after planting, while with high temperatures and "long day" exposures (plants exposed to daylight for thirteen to fourteen hours) flower production takes place forty-seven days after planting. With low temperatures, "short day" plants (i.e., plants exposed to ten hours' daylight) vegetated actively for 116 days before staminate buds were produced, and "long day" plants (i.e., plants exposed to daylight for thirteen to fourteen hours) remained vegetative for a minimum of ninety-two days. The "long day" plants showed an increase in height (25 cm.) over the "short day" plants (11.5 cm.), and with twenty-four hour illumination the plants reached a height of 40.5 cm.

Although at the present time a great many facts have been collected regarding the effect of the duration of light on plant

where  $W_1$  is the final weight,  $W_0$  the initial weight,  $r$  the average rate of interest,  $t$  the time, and  $e$  the base of natural logarithms. Blackman considered that  $r$  is an important physiological constant. It represents the efficiency of the plant as a producer of new material, and he termed  $r$  the "efficiency index" of the plant. From a consideration of this conception two factors are necessary for the best production of vegetative material by an annual plant: (1) a large seed, and (2) a high economy of working represented by a large efficiency index. It is clear that a good start means a larger capital to work on, for the efficiency of the plant is highest in its earliest stages of growth.

G. E. Briggs, Kidd and West (1920) considered that a rigid application of the compound interest law throughout the life of the plant is impossible, and that  $r$  is not a constant at all. But, as Blackman (1920) has himself stated, these workers are under a misapprehension regarding the nature of  $r$ . The value  $r$  must be taken as an average, not as a rigid constant in the same sense as a physical constant.

Blackman's views on growth are not universally held, and a second group of workers, especially prominent in America, consider growth to be of the nature of an autocatalytic reaction. As the name implies, an autocatalytic reaction is a reaction capable of self-catalysis, one of the products of the reaction acting as a catalytic reagent. An example of an autocatalytic reaction may be found in the hydrolysis of esters by water. The monomolecular reaction between ethyl acetate and water to give acetic acid and ethyl alcohol:—



is one such case. The reaction at first proceeds slowly and gathers velocity with increase in the concentration of the acetic acid split off in the course of hydrolysis. The acetic acid here acts as the catalytic reagent, for the presence of acids markedly favours hydrolysis of esters. The curve for such a reaction is typically S-shaped.

From the similarity in the shape of the curves, Robertson (1906) advanced the view that growth is of the nature of a monomolecular

growth and development, yet the underlying physiological causes of this behaviour remain obscure. It is only within the last few years that any chemical investigations have been made in an endeavour to ascertain the fundamental causal relations between duration of light and the behaviour of plants. A number of investigators have pinned their faith to the carbohydrate/nitrogen ratio (see Chapter VII) as being the universal panacea which will extricate them from the difficulties of the position and in this country, Tinckner (see below) has been an active advocate of this particular explanation.

It is generally accepted that the appearance of the reproductive organs heralds a change in the metabolism of the plant. In *Xanthium pennsylvanicum*, Gilbert (*ibid.*) found that the onset of reproduction in "short day" low-temperature plants brought about an increase in the C/N ratio. In the seedlings, on the other hand, whether germinated at high or low temperatures, there is a marked accumulation of reducing sugars.

By treating plants with continuous electric light for a few days, or increasing the daily illumination period for more than twelve hours, Klebs (1918) was able to force *Sempervivum*, which normally flowers in June, to flower in winter. Light here appears to be essential for the formation of the flower primordia.

Pfeiffer (1926) has found in *Mirabilis Jalapa*, the tomato, and buckwheat, that short exposures to light tend to give low protein and carbohydrate reserves and less production of differentiated tissue than longer exposures to light; while with still longer exposures there is an increase in the carbohydrate, but no corresponding increase in protein and tissue production.

Winter wheat planted in the autumn lives through the winter in a dormant or resting condition with no apparent stem elongation and with the leaves appressed to the ground; the so-called "rosette" stage of growth. In the following spring the stems elongate and the plant becomes erect, and normal heading and grain production takes place. On the other hand, spring-sown winter wheat does not pass through the "rosette" stage but grows erect from the first, and heads abnormally if it heads at all. According to Hurd-Karrer (1930), who grew a winter wheat

(Turkey) under greenhouse conditions in which the temperature could be controlled, and in two different light intensities, 50-100 candle-power and 15 candle-power, at relatively low temperatures ( $10^{\circ}$ - $12^{\circ}$  C.) and under long periods of illumination (sixteen and a half hours), the plants left the rosette habit early and became erect. At higher temperatures ( $20^{\circ}$  C.) this wheat did not continue in the rosette state even under short days (eight to nine and a half hours). She is of the opinion that this rosette stage is a true resting period, and if not essential, it at least plays an important part in the subsequent normal growth and maturation of the plants. Forster, Tinckner, Vasey and Wadham (1932) are not in agreement with this view and claim that in England at lower temperatures than  $10^{\circ}$  C., slow vegetative growth still continues slowly in winter and more quickly in spring and summer, while they could find no evidence that short periods of light produce a resting stage.

It has been known for some time that varieties of wheat which are satisfactory in Australia as regards development are failures in England. They give low yields and show poor development and usually appear as early varieties. In the same way, English varieties grown in Australia also behave in an unsatisfactory manner and tend to be late. They produce too many tillers and their heads are formed too late in the season to avoid the hot, dry summer. Forster, Tinckner, Vasey and Wadham (1932) have now made an extensive investigation on the subject. Australian varieties were grown in England and English varieties in Australia in an effort to ascertain whether length of day determined this result. The length of day in Victoria (Australia) does not exceed fifteen and a half hours, whereas in England it may be of eighteen hours duration. The English varieties grown in Australia were on this account treated with extra artificial illumination to bring the daily period of illumination up to that experienced by plants growing under similar conditions of cultivation in England six months later.

The Australian varieties tested proved capable of exerting spikes under shorter periods of light than did the late flowering British spring varieties. Since the duration of light in Australia is shorter than in England, the comparative failure and general

unsuitability of the British varieties for Australian conditions may be partly explained by these experimental results. A connection was observed between the length of the period of light and the longevity of the plant. In this respect wheat falls into Garner and Allard's class of "long day" plants. Vegetative development, of which the number of tillers frequently provides an index, was prolonged by "short days." Leaf development was continued far into the season, and in certain cases it was continued even after flowering and ripening of the grain, in a manner comparable to the aftermath growth of grasses.

Tincker (1928), using various plants of economic importance, such as *Lespedeza stipulacea*, *L. striata*, *Clarkia*, *Godetia*, *Hordeum vulgare* and *Senecio vulgaris*, among a large number of others, found that the influence of duration of light varied considerably in its effects, and the extensive results obtained must be sought in the original memoir. Tincker, however, has attempted to correlate his facts by a chemical investigation under the various conditions of light duration that were used in these experiments. In *Helianthus tuberosus*, for example, shortened periods of daylight caused intense tuberisation. The manufactured carbohydrates passed down the stem to the tubers, which were not rich in protein or inulin compared with the controls. In the latter the stem contained more inulin, since elongation requires adequate supplies of carbohydrate. It would therefore appear that the duration of the illumination has a controlling influence upon the translocation and utilisation of the products of carbon assimilation. With *Phaseolus multiflorus*, exposure to short periods of daylight did not result in stem elongation. The short stems were found to be rich in starch and bore thick, tough leaves with long palisade cells. Moreover, the roots showed secondary thickening, and also contained much starch in the wood parenchyma. Grasses, such as *Phleum pratense*, *Dactylis glomerata* and *Anthoxanthum odoratum*, were prevented from flowering in one season by exposure to short periods of light, but when exposed in a subsequent season to natural conditions of illumination they flowered earlier than the controls.

Although considerable attention has been paid to the effect of

photoperiodism on the development of the shoot and reproductive organs, comparatively little work has been carried out with respect to the development of the root system under varying durations of light. Garner and Allard (1923) stated from some preliminary observations they had made that the duration of the daily illumination period may exert a marked effect on the relative development of the root and aerial portions of the plant. They found, for example, that a cutting of Biloxi soy-bean made no top growth at all during the winter months and the original leaves showed a dark green colour. Seemingly no new buds could develop. When the root system was examined in the spring it was discovered that the soil contained a large mass of roots which were altogether out of proportion to the size of the plant as judged by the standard of the usual summer growth. The growth of root and shoot, therefore, is not necessarily contemporaneous as far as season is concerned, and arrested development of the aerial portion of the plant, which is exposed to suboptimal light duration, need not be accompanied by checking of root growth.

According to Crist and Stout (1929), lettuce and radish exposed to shortened periods of illumination (six hours daily) give a greater ratio of tops to roots, based on dry weight, than plants exposed to normal daily illumination in cloudy winter weather, or when the light duration is prolonged a further period of six hours by means of artificial illumination. It was also ascertained that the plants grown under the longer period of illumination had the lowest top-root ratios, while the "short day" plants had the lowest actual weight of both tops and roots.

Weaver and Himmel (1929) have carried out an extensive investigation on the effect of duration of light on the development of the root system of a variety of plants, such as *Trifolium pratense* (red clover), *Raphanus sativus* (white icicle radish), *Iris germanica*, *Helianthus annuus*, *Dahlia pinnata* (yellow duke dahlia), *Ambrosia trifida* (great ragweed), *Avena sativa* (white Kherson oat), and an early flowering cosmos (*Cosmos bipinnatus*). The plants were grown in heavy galvanised containers filled with a rich loam soil of optimum and uniform water content.

The plants were exposed to under seven hours of daily illumina-

tion (short period) and fifteen hours' light duration (long period) and the relative development of roots and tops determined.

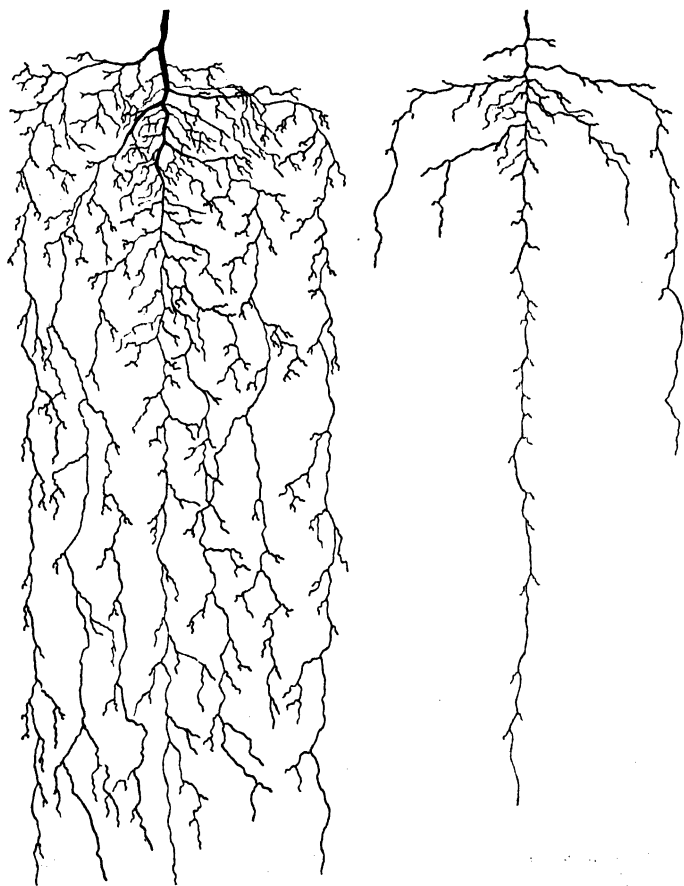


FIG. 52.—Relative development of root systems of red clover under 15 hours' (left) and seven hours' daily illumination. (After Weaver and Himmel, *Plant Physiol.*)

Of the various species employed in this work, *Trifolium pratense*, *Raphanus sativus*, *Iris germanica* and *Avena sativa* belong to Garner and Allard's category of "long day" plants,

while *Dahlia pinnata*, *Ambrosia trifida* and *Cosmos bipinnatus* belong to their "short day" group. The time of flowering of *Helianthus* appears to be less modified by the length of day than the "long day" group.

As a result of shortened daily illumination, the "long day" plants showed considerable retardation of both shoot and root development. Fig. 52 shows the development of the root system of *Trifolium pratense* under the two periods of illumination used in this work. Similarly, the "short day" group also attained its greatest development of shoot and root under the longer period of illumination (fifteen hours). When exposed to seven hours of light daily the dwarfed tops were furnished with a correspondingly meagre root system.

The data presented shows that in every case development of the absorbing system was clearly correlated with the transpiring surface. Measurements of photosynthetic activity were carried out by determining the amounts of total carbohydrates in the leaves as well as increase in carbohydrate content after insolation. It was found that in the "long day" plants there was either increase in photosynthetic activity, or alternatively, less activity in removing or using the product under "long day" illumination than under a seven day, while in the "short day" plants used in this work the amount of photosynthetic activity was more or less equal under the two conditions of illumination, or alternatively, they were less active in removal or use of the products under "short day" illumination.


Zimmerman and Hitchcock (1929) have discovered that the length of day determines the type of root system formed by dahlia cuttings. "Short day" illumination results in heavy root-storage, while "long day" illumination gives rise to a fibrous root system. There is an accumulation of nitrates both in the leaves and the stems of "short day" plants, whereas "long day" exposure either entirely or markedly reduces the nitrates present in these organs. Generally it was ascertained that flowering was independent of storage root formation, although, in two varieties (Jersey Beauty and Mrs. I. de ver Warner) flowering and storage root formation occurred concurrently with "short day" exposure.

Others (Arthur and Summer Red) flowered independently of day length, while others again (Jersey Beauty and Warner) flowered only with "short day" exposure.

The majority of investigations concerned with photoperiodism have been conducted with the natural illumination of sunlight. It is obvious, however, that only controlled conditions of light intensity and duration will reveal the underlying cause of the phenomenon and help in its explanation. A few such investigations have been recorded and others are in the course of progress.

Shirley (1929) grew a range of plants under constant conditions of temperature in sunlight and artificial light. It was found that for the large range of plants used, which included *Geum canadense*, *Fagopyrum esculentum*, *Lycopersicum esculentum*, *Nicotiana Tabacum*, *Zebrina pendula* and *Helianthus cucumerifolius*, the intensity of the light required for survival was low, being less than 40 foot-candles for all the plants with the exception of *Helianthus cucumerifolius*, which needed a considerably higher value. The dry-weights produced by these plants at these low light intensities were almost directly proportional to the intensity received, up to about 20 per cent. of full summer sunlight. As the higher intensities of light were reached, direct proportionality was no longer exhibited and the slope of the curves fell away; the shade plants showing a decrease at lower intensities than sun plants. The flowering and fruiting time was considerably delayed with low intensities of light, and, in fact, fruiting did not occur at all with plants grown in light intensities below 8 per cent. of that of full summer sunlight.

Ashby (1929) has made an important investigation on the effect of light duration on *Lemna minor* under controlled conditions of temperature and light intensity. The plants were grown in special growth chambers, whereby four colonies could be investigated simultaneously for different periods of illumination. The colonies were exposed to artificial light for periods of twelve hours, six hours, and also two-hour alternate periods. With the exception of the experiments conducted at light intensities of 700 and 800 foot-candles for six hours' duration, the curves obtained were all of the exponential type. In the case of the light



intensities of 700 and 300 foot-candles for six-hour periods the values lay rhythmically about a straight line. In general terms, the growth increased with increase of light intensity up to 700 foot-candles, but showed a considerable fall at 1,400 foot-candles. At the latter intensity the light had a definitely harmful effect upon the plants.

Ashby made the suggestion that after the optimum of light intensity is reached (the optimum being somewhere between 700 and 1,400 foot-candles), the reduction in the rate of growth at supra-optimal light intensities is probably due to a reduction in the chlorophyll content of the fronds. There is, however, some other detrimental factor concerned as well, since with reduction in the periods of light there is a reduction in the growth rate without any corresponding reduction in the amount of chlorophyll in the fronds.

Tincker (1928) considered that the duration of light affects the carbohydrate/nitrogen ratio (see Chapter VII) and found that there is a considerable correlation between chemical composition as expressed by this ratio and the behaviour of the plant. "It would seem that the length of day influences the rate of elongation of the stem, and controls the utilisation of the photosynthetic compounds. By this means the carbohydrate/nitrogen ratio of the tissues is influenced. In general there would appear to be a correlation between the carbohydrate/nitrogen ratio and the behaviour of the plant. This does not necessarily signify that the magnitude of the ratio determines the behaviour of the plant and the nature of the growth made—the reverse may equally well be the true interpretation of the facts."

One particular difficulty that confronts any general employment of the carbohydrate/nitrogen ratio to the facts of photoperiodism lies in the distinction that must be kept between "long day" and "short day" plants. The application of the C/N ratio to explain the reaction of "long day" plants is, on the surface, not unreasonable, since it may be supposed that the abnormal light period increases the amount of carbohydrate material manufactured in carbon assimilation, and in this way a correct C/N ratio is established at an earlier stage than would be otherwise possible.

Difficulties, however, arise in the case of "short day" plants. Here retardation of light duration accelerates flower production and the question arises as to how this can be fitted into any explanation depending on the C/N ratio. How is it possible in these circumstances to obtain the correct ratio, since short duration of light must adversely affect the amount of photosynthate formed?

Nightingale (1922, 1923), working with *Salvia*, buckwheat and soy-bean, found that plants illuminated for seven hours each day had a higher percentage of nitrates and carbohydrates than "long day" plants. He considered that the carbohydrates accumulated in the "short day" plants, presumably because there is relatively little utilisation of them in the conversion of nitrates to other forms of nitrogen.

According to Reid (1929), nitrates are synthesised into growth-promoting substances both in light and darkness, but the synthesis takes place much more rapidly in the light. The nitrates favour the growth of the shoots more than the roots, while the light favours the growth of the roots. Seedlings with low nitrogen reserves undergo rapid differentiation and maturation of tissues in light, and the lower the nitrogen content of the seedlings the more rapid is the process. The relative proportion of shoots to roots varies with the season of the year. In the "long day" months the ratio is relatively low, and in the "short day" months it is higher.

A different explanation to account for plant behaviour under varying periods of light duration has been advanced by Redington (1929). The species used in this work were grown entirely in artificial light, and daylight was not employed at all. The plants were exposed to (1) continuous light for the whole of the growth period, (2) sixteen hours of light and eight hours of darkness, and (3) eight hours of light and sixteen hours of darkness per day. The following were among the species investigated: *Pisum sativum*, *Phaseolus sativa*, *Gypsophila elegans*, *Galium verum*, *Linum usitatissimum*, *Humulus japonicus*, *Cannabis sativa*, *Salvia splendens*, *Manihot*, *Cucurbita Pepo*, *Pelargonium* (sp.), *Kleinia obtusifolia*, *Zea Mays*, *Gossypium herbaceum* and *Fagus sylvatica*.

All the species which flowered under the experimental conditions (these included *Pisum*, *Vicia*, *Gypsophila*, *Galium*, *Linum*, *Humulus*, *Gossypium*, *Pelargonium* and *Zea*) did so earlier under continuous light than in sixteen hours' light per day, and only one species, *Pisum*, produced flowers in eight hours' light per day. The quantity and development of the flowers produced were generally adversely affected by conditions of continuous illumination, for in all species except one (*Pelargonium*), flowering was more profuse and seed production greater under conditions of intermittent light. With regard to vegetative growth, three groups of plants could be distinguished according to their condition at the end of the experimental period: (a) Species which made noticeably more growth in continuous light than in intermittent light (*Fagus*), (b) species which grew reasonably well in continuous light, but which made much better growth with a rest period of eight hours' darkness per day (*Pisum*, *Vicia*, *Gypsophila*, *Gossypium* and *Zea*, among others), and (c) species which made very poor growth in continuous light (*Kleinia*).

✓ It was found that practically all the plants exposed to continuous light grew at a more rapid rate in the early stages of development than those exposed to intermittent light, but sooner or later this abnormally vigorous rate of growth lessened, with the result that in all species, with the exception of *Fagus*, bigger plants were produced with sixteen hours of light per day. It was also discovered that the plants grown in sixteen hours of light were usually taller, a result due to the greater average internode length. A constant feature of the plants grown under continuous illumination was the rapidity with which the leaves became yellow and withered and fell after reaching maturity. Their average size was also less than that of similar leaves grown in sixteen hours of light.

The species grown under eight hours of light per day could be divided more or less into two groups, according to their behavior under these conditions. One of these groups made vacuumer plants (*Gypsophila*, *Galium*, *Linum*, *Cucurbita* and *Ficulis*, while the other gave plants which attained a good height under yellow light, appearance, externally at any rate, compared with the blue-violet

autocatalytic reaction and that some special catalyst of the nature of an enzyme governs the growth rate of an organism. Robertson, from his experimental results, supposed that in any particular growth cycle, either of the organism as a whole or of any part of the organism, the greatest increase of volume or weight in any unit of time takes place when the total growth due to the cycle is half accomplished. Such a growth-cycle is said to conform to the equation :—

$$\log \frac{x}{A-x} = K(t-t_1)$$

where  $x$  is the amount of growth in weight or volume which has occurred in the time  $t$ ,  $A$  is the total amount of growth attained during the cycle,  $K$  is a constant, and  $t_1$  is the time in which the growth-cycle is half completed.

Certainly Robertson's figures expressed in the form of a curve show an excellent agreement with such an expression, although, when expressed as a percentage, they are too large to support his views.

If growth were simply due to the result of an autocatalytic reaction, once it had attained its maximum point a constant value should be recorded. This is not the case in practice, and, with advance in time, a loss is found. This is considered due to secondary changes.

Reed and Holland (1919) strongly supported Robertson in this respect. They found that the growth rate of the annual *Helianthus annuus* approximated to that of an autocatalytic reaction in which the growth rate at any particular time could be expressed by the equation :—

$$\frac{dx}{dt} = Kx(a-x)$$

where  $a$  is the initial quantity of material subject to transformation,  $x$  is the amount transformed at a time  $t$ , and  $K$  is a constant. According to these investigators, the growth rate is governed by constant internal factors rather than external factors.

Again, Reed (1920) found that the growth rate of apricot shoots followed a definite but fluctuating rate. The maximum rate was shown soon after the season's growth had begun ; but at the same

to light of longer duration. Plants exposed to eight hours of light generally showed stems which were thinner and internodes fewer and longer than plants which had been exposed to sixteen hours of light. The leaves were also smaller and the petioles longer.

Redington has put forward the following hypothesis to account for the behaviour of these various plants under different periods of illumination. He pointed out in the first place that growth in the flowering plant (and by growth here he presumably means stem elongation and not increase in dry weight) is the result of two distinct processes : (a) Formation of new cells at the meristem and (b) subsequent differentiation and elongation of these cells. In the majority of dicotyledonous plants the tissue mainly responsible for growth in length of the stem is the apical meristem. "The synthesis of protoplasm by the meristem is dependent upon the free supply to it of organic nutrients, whose carbohydrate nuclei are built up in photosynthesis and subsequently combined with nitrogenous and other inorganic substances taken in by the roots to form the 'bausteine' of protoplasm. The relatively impermeable walls of the meristem which form the ultimate supply channels to the synthesising protoplasts cannot be freely irrigated with these raw materials of growth if a condition of water strain exists in the vascular system as a result of excessive transpiration, and if they and the cell walls of the adjoining vacuolating tissue are directly subjected to the excessive drying action of continuous light exposure.

"The subsequent vacuolation and elongation of these cells will be governed by the supply of water to them, by the maintenance of the necessary osmotic pressure as a result of the presence of osmotically active substances in their sap, and by the resistance offered by the young cellulose cell wall to the internal hydrostatic pressure. In continuous light we may reasonably expect a falling of the water supply to such tissue, a leakage of salts via the protoplasmic membrane, whose permeability has been increased as a result of the abnormal light exposure, and a consequent loss of turgor. The time during which the cell walls are in the 'amyloid' state, the maximum extensibility."

The question arises as to how far this hypothesis can be applied to plants growing under natural conditions. Redington's material was grown under artificial conditions. The explanation advanced by him takes a number of matters, which are still in a controversial state, as being proved facts. A great deal of play is made about the permeability of the cells at the meristem, but as the whole subject of the permeability of plant cells is in a deplorable state, it seems somewhat premature to make a number of sweeping assumptions based on insufficient data with regard to the sequence of events in plant meristems and the subsequent behaviour of the plant.

### **The Effect of different Parts of the Spectrum on Growth**

The various parts of the spectrum have very different effects upon the growth and reproduction of plants. Many of the older experiments on this subject are vitiated by the fact that no account was taken of the energy relations involved. It is obviously necessary to have particular regard to the fact that, in experiments of this nature, due care is taken that the same energy relations prevail in all the experiments. If, for example, plants are being grown under blue and red screens to discover the action of blue and red rays on growth, the energy values of the two kinds of glass must be the same; should the blue glass absorb more light energy than the red, no comparison can be made between the effects of the blue and red rays on growth.

Schanz (1919) grew plants under glass which transmitted definite parts of the spectrum. Eight beds were covered with different coloured glasses. In the first five beds the light transmitted passed from violet to red, and in the final three beds combinations of glasses allowed yellow, green and blue-violet light to pass through. He found that taller plants were produced when the short rays of sunlight were removed. The maximum height was obtained under the red and the minimum under the blue-violet rays. This was found to be true for cucumber, *Fuchsia*, *Chrysanthemum*, *Lobelia*, *Begonia* and *Oxalis*, while potatoes and beet showed the weakest growth in yellow light, stronger in green, and still larger and healthier in the blue-violet

region. The development of chlorophyll in soy-beans, beans and potatoes was more rapid when the short rays were cut off, and the most rapid development took place in the red rays. With lettuce the chlorophyll was not produced in normal amount in yellow or green light but developed fully in violet. It appeared from Schanz's experiments that ultra-violet light was necessary for the development of anthocyanin pigments, for even the flowers were pale in colour if the very short rays were removed. The time of flowering was hastened in *Fuchsia*, tomatoes and beans, and the number of flowers and fruit produced was increased as the short wave-lengths of light were cut off. With red, yellow, green and blue-violet rays the number of flowers was reduced and the time of flowering postponed. The general opinion of this author is that the short wave-lengths of light are detrimental to growth, especially the rays in the blue-violet region.

Popp (1926) has considerably extended these observations. A number of different species were grown in five specially constructed greenhouses, which transmitted the following parts of the spectrum :—

- House (1).—From red end of spectrum to  $312\mu$ .
- „ (2).—All wave-lengths to  $296\mu$ .
- „ (3).—Ultra-violet rays eliminated.
- „ (4).—All rays shorter than  $472\mu$  eliminated.
- „ (5).—All rays shorter than  $529\mu$  eliminated.

A wide variety of plants was used in these experiments, which included, among others : *Nicotiana Tabacum*, *Daucus Carota*, *Petunia hybrida*, *Helianthus cucumerifolius*, *Soja max*, *Mirabilis Jalapa*, *Coleus Blumei*, *Fagopyrum vulgare*, *Lycopersicum esculentum*, *Holcus* (sp.) and *Sorghum sudanensis*.

The most striking results were obtained in houses (4) and (5), in which the entire blue-violet rays were eliminated. The plants showed decided signs of etiolation, although a good green colour, and in some cases (tomato) an even greener appearance was shown than by plants in the other houses. The stems were tall and slender, the internodes greatly elongated, while soy-beans (*Soja*

*max*) changed their habit of growth and became climbers. The leaves showed a tendency towards crinkling and rolling, which was pronounced in the case of *Helianthus cucumerifolius*, *Petunia hybrida*, *Nicotiana Tabacum* and *Mirabilis Jalapa*. There was considerable development of loose palisade tissue in the leaf, except in the soy-bean, which showed a considerable delay in reaching maturity compared with the plants in the other houses.

The removal of the ultra-violet rays, house (3), had little effect on the general growth and appearance of the plants. They were perhaps slightly taller and bloomed earlier than in houses (1) and (2). Plants in houses (1), (2) and (3) possessed a vigorous and healthy appearance. The rate of germination was unaffected in any of the houses. In the first two or three weeks stem elongation was greatest in houses (4) and (5), but later the rate fell below plants in the other houses. The removal of the blue-violet end of the spectrum uniformly resulted in decreased stem thickness; the smallest stems occurred in houses (1) and (2). The removal of the blue-violet rays also had a marked effect in some species on the flowering time; plants in houses (4) and (5) uniformly flowered later than in the other houses, except soy-beans, while sunflowers practically failed to flower and the number of flowers produced in the other species was greatly reduced. The dry-weight of the plants was lowest in houses (4) and (5), and the percentage of moisture greatest. A chemical analysis of the plants showed that the greatest amount of nitrogen and the lowest percentage of total carbohydrate occurred in houses (4) and (5).

The results of these experiments indicate very clearly that blue-violet rays are necessary for good and healthy growth. The absence of rays shorter than  $529\text{ }\mu\mu$  results in more or less etiolation. The intensity of the light is not an important factor in this connection, for normal growth is obtained when the plant receives the full spectrum of daylight at an intensity which at all times is less than that of the house in which all wave-lengths shorter than  $427\text{ }\mu\mu$  are removed, and only slightly greater than that of the house in which wave-lengths shorter than  $529\text{ }\mu\mu$  are eliminated.

### Polarised Light and Growth

It was suggested in 1924 by Baly and Semmens that polarised light markedly influences the hydrolysis of starch and that starch is very much more readily hydrolysed by diastase in the presence of polarised light. This statement was contradicted by Neilson Jones (1925). Later, Macht (1926) stated that polarised light had a marked action upon certain drugs, while Bhatnager and Mathur Lal (1926) claimed that polarised light had a specific effect on some chemical reactions and the growth of micro-organisms. Nevertheless, other workers, *e.g.*, Bunker and Anderson (1928), and Navez, Albert and Rubenstein (1928), have failed to discover this stimulating action of polarised light.

According to Semmens (1930), the leaves of *Tropaeolum*, *Geranium* (*Pelargonium*?) and spinach, when placed under a Nicol prism and illuminated with polarised light in this way and subsequently tested with iodine in potassium iodide for starch, showed clearing of the area under polarised light compared with the rest of the leaf. Apparently it is only young and healthy leaves that show this reaction. In the case of plants with hairy leaves, the hairs depolarise the light and only a faint reaction is given. Semmens claimed that this action of polarised light has a bearing on growth. She grew *Fuchsia* in special chambers, one of which received a diurnal succession of polarised light and darkness, and the other ordinary light and darkness. The plant which received polarised light began to show signs of distortion and starvation.

Dastur and Asana (1932) have studied the effect of polarised light on the rate of photosynthesis in an attempt to confirm Semmens' statements. It was found that the rate of photosynthesis in polarised and ordinary light was identical in *Allium Cepa*. The test here is not quite fair, as *A. Cepa* forms no starch in its leaves, but in two species (*Helianthus annuus* and *Raphanus sativus*) with starch-forming leaves the same result was obtained, and no statistically significant difference could be discovered in the rate of assimilation which proceeded as regularly and vigorously in polarised light as ordinary light.

### Light and Reproduction

It has been known for a number of years that the factors governing vegetative growth are antagonistic to those concerned with reproduction. It was supposed at one time that special internal factors governed the formation and development of reproductive organs. It remained for Klebs to demonstrate that the phase of reproduction is governed by certain definite external conditions and that reproductive structures do not arise from internal causes alone, although these no doubt play an important part. The influence of light on flower production has already been considered for the higher plants; it remains now to discuss the influence of light on some of the lower plants, as well as the effect of light duration on the sexual expression of certain unisexual higher plants, e.g., *Zea Mays* and hemp.

Barnes (1924, 1925) has shown that a species of *Lachnea* has a conidial stage which falls into the genus *Acmosporium*. The appearance of the sexual or asexual stage is dependent on the nature of the medium employed and also on the amount of light supplied to the cultures.

Light has very definite formative influences upon the fungi. Many will not fruit in the dark, while others will not fruit in the light. W. Robinson (1926) has shown that the growth and development of the discomycete, *Pyronema confluens*, is conditioned by a number of separate factors which to some extent interact and are dependent upon one another. The effect of the absorption of light energy is only one such factor in the development of the reproductive structures and apothecia, if and when the mycelium is in a suitable condition for the reception and utilisation of this energy. Although important in themselves, the factors concerned are only one portion of the matter; though they must be favourable, yet a sequence of causation can be recognised. The first stage in the sequence is shown by the definite arrest of development of growth in the main hyphæ of the mycelium, followed by a development of the lateral branch systems which grow into the air, owing to the spacing conditions on the agar surface. Here, moisture relations are altered, and the effect of the energy from

the light is shown in morphological changes accompanying the development of the antheridia and oogonia. The pink pigment, characteristic of the fungus, appears at the initial stage of these changes and increases in amount with development. Robinson was unable to ascertain whether the appearance of the pigment and the development of the reproductive structures were causally connected. He considered that if some pigment had not been formed, the dependence of both upon light and the non-appearance of the reproductive structures was certainly significant.

The aerial branches have the potentiality of developing into reproductive structures before they have received energy from light. This is said to explain the appearance of the abortive structures which arise in darkness in equivalent positions to apothecia in normal cultures. The light operates relatively late upon regions of the mycelium when the potentiality for development has already been determined. The absorption of a certain amount of energy from light is therefore a final phase in the sequence of causation concerned in development. Carbon dioxide and excess of carbohydrates inhibit lateral branch structures. Again, humidity relations must be correct before development of oogonia and antheridia takes place. Only a small quantity of nitrate and carbohydrate is necessary for vegetative development, but reproduction only occurs in fluid cultures when the available supplies of nitrogen are becoming exhausted. There is no general development of reproductive structures if the initial concentration of carbohydrate (here maltose) in the medium be higher than  $M/250$ . Moreover, the moisture content of the air over the cultures exercises an important effect on the reproductive organs. The most favourable conditions for reproduction are found between relative humidities of 50 and 70 per cent. No antheridia, oogonia or apothecia are formed below 15 or near 100 per cent. relative humidity.

The action of different rays of light on the rate of reproduction of *Volvox aureus* and *Closterium acerosum* has been investigated by Klugh (1925). He found that in blue and red light of the same energy value, reproduction was greater in the case of red light. Green light was quite ineffective in this connection.

Wann (1925) has studied the effect of duration of light on the formation of the sexual organs of the bryophyte, *Marchantia polymorpha*. Cultures were exposed to varying periods of daylight to determine the effect of fruiting. The times of exposure were six, eight, ten hours, and full daylight (this was used as the control). A further culture was also exposed to six hours of artificial light from a 10-watt lamp. The cultures exposed to light duration of six, eight, ten hours' daylight and six hours of artificial light all remained sterile from October to February, but the control culture (*i.e.*, the one exposed to full daylight) began to show signs of fruiting and the fruit branches were mature by the end of January, although it was started into culture later than the others (November). It was also found that by increasing the period of light in the other cultures they could be made to fruit earlier. In this respect *M. polymorpha* falls into line with the so-called "long day" plants among flowering plants.

Schaffner (1927) has found that *Zea Mays* definitely influenced its sexual expression by the length of daily illumination. The reversal of the tassel (male inflorescence) to the carpellate form is to be regarded here as an ecological fluctuation depending on a complex of environmental factors. With the correct moisture, nutrient supply, temperature and dimness of light, the amount of reversal, both in respect of population as a whole, and the degree to which it is expressed in any individual, is proportionate to the period of daily illumination. It is, however, the intensity of the illumination which is the deciding factor. Corn, for example, planted on November 1st in the greenhouse will, with correct substratum and heat, show 100 per cent. of individuals with some degree of female expression in the tassel, whereas, if planted in the spring or summer, it will show only pure staminate tassels. If the corn be planted after this date, under similar external conditions, sex reversal is inversely proportional to the length of daily illumination. With the onset of equal day and night periods of twelve hours each, little or no reversal occurs in the tassel. According to Schaffner (1930), with proper photoperiodicity, seven general types of tassels can be recognised in *Zea Mays*: staminate, carpellate, vestigial (neuter) and sex-mosaics, staminate-neuter, carpellate-

neuter, staminate-carpellate and staminate-carpellate-neuter. Staminate character can be completely suppressed in certain circumstances and whole plots developed without the appearance of a single stamen. The whole matter is entirely dependent upon the duration of light. Schaffner's conclusions are somewhat dogmatically stated, but he considers that little value can be attached to the deductions from genetical studies carried out to discover hereditary potentialities unless proper account is taken of the ecological-physiological control of hereditary expression. In the case of hemp, light in certain conditions is able also to cause sex reversal. Schaffner (1923) exposed hemp to short periods of daily illumination, when a considerable amount of sex reversal took place; about 90 per cent. of male and female individuals. With longer periods of illumination no reversal occurred. He concluded that the light must produce some effect on the hemp besides supplying the necessary energy for photosynthesis.

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time three intra-seasonal growth-cycles were exhibited. The growth rate in each cycle closely followed the rate of an autocatalytic reaction. The growth rate for the entire season conformed to that of a reaction consisting of two monomolecular reactions, one of which at first accelerated and later retarded the other. Reed measured the rate of increase in height of walnut trees. The young trees showed distinct cycles of growth in a single season, but in each cycle the rate of growth conformed to an autocatalytic reaction. Reed (1921) has emphasised the fact that a relation exists between the rate of growth and the final size of the plant. The amount of growth yet to be made appears to be an essential function of the final size. The rate may be affected by two factors: (1) a variation in the supply of catalysts; and (2) a variation in the supply of potential growth material.

The "summation curve" (the total number of flowers up to a given date) in the case of the Egyptian cotton plant has been found by Prescott (1922) to be typically S-shaped and can be expressed by the equation:—

$$\log \frac{x}{a-x} = K(t-t_1)$$

where  $a$  is the total number of flowers obtained,  $x$  is the number of flowers up to a given time  $t$ ,  $t_1$  is the time when  $x = \frac{a}{2}$ , and  $K$  is a constant. In other words, the summation curve appears to follow an autocatalytic reaction.

Mitscherlich (1919) has evolved the equation:—

$$\log (n\sqrt{A} - n\sqrt{y}) = \log (n\sqrt{A} - cn)$$

to express the growth of a plant, in which  $n$  is a variable quantity indicating the probable number of external factors,  $A$  is the maximum possible dry-weight attainable by the plant in question,  $y$  is the dry-weight of the plant at a time  $x$  ( $x$  being expressed in vegetation periods of any arbitrary length) and  $c$  is a constant. It is doubtful if such an expression has any significance in practice. An equation of this kind may fit the facts very well in one case, yet be found to be wanting in another. Rippel (1919) strongly criticised such an expression and considered that Robert-

## CHAPTER IX

### GROWTH (*continued*)

#### ACCESSORY GROWTH FACTORS AND RELATED PROBLEMS

*Auximones—Bios—Hormones—Phototropism and the Growth Regulator of the Coleoptile—Geotropism and the Action of G.R. upon the Root—The Chemical Nature of G.R.—Growth and Inhibition.*

A NUMBER of subsidiary factors concerned in plant growth and development can be conveniently considered here. Prominent among these we have the auximone problem, the bios question, and the subject of hormone activity in the plant economy.

#### **Auximones**

Bottomley (1917, 1920) suggested as a result of a number of observations he had made on the growth of the aquatic ferns, *Salvia* and *Azolla*, as well as the common duckweed, *Lemna minor*, that, in addition to the ordinary culture medium (Detmer and Knop) made up of mineral salts, there was needed another factor for successful growth, and to this he gave the name *auximone*. By employing aquatics for this work, Bottomley did away with the artificial conditions of growing normal land plants in culture solution. In all cases he found that it was necessary to add organic matter to obtain healthy plants, and claimed that aqueous extracts of bacterized peat and material containing nucleic acids gave the best results. The amounts necessary to bring about successful growth and division were said to be minute, and these growth-promoting substances were considered to have a resemblance to the vitamins of the animal biochemist. Mockeridge (1920, 1924) supported Bottomley's views on this matter, and Rosenheim (1917) claimed that they held good for the successful growth of *Primula malacoides*.

Bottomley's ideas on the necessity for subsidiary growth factors or auximones have not met with universal acceptance. N. A. Clark and Roller (1924) have shown that certain lower plants can be grown for months on end in a solution of purely inorganic salts, provided that the physiological balance of the solutions be correct. Similarly, Saeger (1925) has been unable to confirm Bottomley's results, and found normal growth of *Spirodela*, *Lemna* and other aquatics in ordinary culture solutions in the absence of additional organic matter, provided that the solutions were diluted ten times. Wolfe (1926) has confirmed the work of Clark and Roller and Saeger, and has gone so far as to suggest that the term "auximone" should now be dropped from the literature. Mockeridge (1927) in a reply to these investigators pointed out that their media may have become contaminated with bacteria, which, she states, have a powerful effect on the growth of *Lemna*, and that it may be on this account they were able to grow these forms for extended periods of time in purely mineral culture solutions.

It has now been shown by Ashby (1929) that the addition of organic matter does have a definite and remarkable influence on the growth of *Lemna*. As Ashby has pointed out, Bottomley and his co-workers made no attempt to control external conditions in their experimental work, especially the *pH* of the culture medium. This latter factor is especially important, as *Lemna* is very sensitive to changes in *pH*. Ashby grew *Lemna* under exactly controlled conditions in dilute culture solutions of mineral salts alone, as well as with the addition of organic matter (aqueous extract of sterilised horse dung). It was found that *Lemna* could grow indefinitely in culture solutions composed solely of mineral salts, but the addition of organic matter produced certain remarkable results. The cultures were grown under constant conditions of light and temperature, and in a constantly circulating current of air. Statistical examination of the data showed that the plants growing in cultures with the addition of horse dung (0.2 parts per million) showed an increase in frond area and cell size over the controls. The number of chloroplasts per frond also increased, resulting in an increased rate of photosynthesis and therefore in an increased

growth-rate. The influence of the organic matter is apparently catalytic in nature, for increase in the amount added in greater concentration than 2.0 parts per million caused no increase in the growth-rate. It seems clear from this investigation that although *Lemna* is able to develop normally without the extra addition of organic matter, nevertheless its presence does have the effect of increasing the growth-rate to a remarkable extent, thus confirming Bottomley's original observations.

N. A. Clark and Roller (1931) are not in agreement with Ashby's results, and although they state that the addition of sterilised organic matter to cultures of *Lemna major* led in their experiments to an increased rate of reproduction, they consider that this result is due to the contaminating presence of micro-organisms. If the plants themselves were first sterilised, they found that there was no increase in the rate of reproduction when organic matter was added. A number of justifiable criticisms can be made about this work. In the first place, the plants were sterilised with either potassium mercuric iodide or bleaching powder, and such treatment appears to be drastic; more especially is this seen to be the case when Clark and Roller admit that only thirty plants out of 400 survived this treatment. Secondly, Clark and Roller themselves say that the addition of organic matter does increase the rate of reproduction, but the result is due to the presence of micro-organisms in the *Lemna* plants, and thereby tacitly admit Mockeridge's answer to their earlier work (see above).

### Bios

In 1901 Wildiers found that when he employed small inoculations of yeast hardly any growth or fermentation took place, but when he used large "seedings" normal growth and fermentation occurred. In the latter case it was assumed that a special chemical substance was present which is indispensable for normal growth of the yeast plant. To this substance the name *bios* has been given. The literature surrounding the whole subject of bios is of a very controversial nature, and its presence has been affirmed and denied several times. A good deal of the uncertainty and difficulty

about the bios problem and yeast growth appears to be due to the fact that certain races of yeast are able to grow and develop normally without the addition of bios to the synthetic medium, whereas others cannot. For very full reviews of this subject the articles by Tanner (1925) and Peskett (1933) should be consulted.

### Hormones

In animal physiology the effect of traces of substances in stimulating in a marked manner various physiological activities, especially those concerned in secretion, has been recognised for many years. These substances are produced in one organ and stimulate another organ to which they are conveyed in the blood stream. The process of secreting particular substances into the blood is called *internal secretion* by animal physiologists, and the various organs which carry out this process are termed ductless glands or *endocrines*, and their study, which has now reached large dimensions, goes under the name of *endocrinology*.

Regulation in the animal body by means of nerves is comparable to a telegraphic system, because particular parts of the body are connected by definite nerve-fibres, and in this way nervous messages are sent only to the particular organs concerned. On the other hand, regulation by means of internal secretions can be likened to a postal system, for although the substance is broadcast through the blood stream it only reacts upon a specific organ. From this fact arose the idea of chemical messengers, or, as these substances are now called, *hormones*. A number of these hormones have been isolated in the pure state and even synthesised in the laboratory.

It was first suggested in 1907 by Errera that hormones probably play a part in the plant economy, and it is now becoming increasingly apparent that hormones carry out important functions in the physiological activities of plants.

One of the best-known examples of hormone activity in a plant is the case of the so-called sensitive plant, *Mimosa pudica*. The pinnate leaves of *M. pudica* are very sensitive to touch, and the leaflets close together in a characteristic manner.\* It was first

shown by Ricca (1916) that the stimulus or hormone involved here passes through the xylem in the transpiration current, and not, as Haberlandt considered, through the phloem. In *Mimosa* it was found by Ricca that if the stem were killed with steam or separated by a water-gap, the hormone was still able to pass in an upward or downward direction. This work has been further extended by Snow (1924, 1925a), who was able to confirm Ricca's original observations and added several of his own. He showed, for example, that though the phloem of the stem was quite insensitive to the passage of the hormone, it was conducted through the phloem in the petiole, a process which he has termed "high-speed conduction." He also discovered that the hormone is thermostable in the plant, but watery extracts are killed by boiling. Snow prepared water extracts of the hormone, and found that it was not precipitated by lead acetate and that it gave none of the reactions of a protein. He was also able to show that it diffuses through a collodion thimble without losing its properties. It is possible that it may have a comparatively simple chemical constitution. Could it be isolated in sufficient amount, a study of its chemical properties would be of the utmost interest.

Ball (1927) has also investigated the conduction of the stimulus in *Mimosa* and has demonstrated that at least two methods of conduction are in existence in the stem. The normal method of conduction is through the xylem, as described by Ricca and Snow, and in this case the stimulus travels at a rate of about 15 to 28 cm. per minute, but in addition there is another method of conduction in which the stimulus travels through the pith at the rate of 200 cm. per minute. This latter rapid conduction method is shown when cut shoots are completely submerged in water. Ball proved quite clearly that the stimulus does not pass through the phloem in the stem, for on decortication and removal of all tissues external to the wood, the stimulus still passed with the same rapidity as before. Under normal conditions the hormone is released at the point at which the stimulus is applied and is transported in the transpiration stream through the xylem, and this method of conduction operates best when the turgor of the cells is low and the water tension in the xylem vessels high.

When, however, the turgor of the tissues is high, the hormone is still released at the point of stimulation, but, instead of passing up the xylem, it merely causes contraction of the neighbouring cells which are possibly situated in the pith. These in turn release a further quantity of hormone, and in this way a relay mechanism of a highly efficient nature is set up, whereby the hormone can pass in either direction in the plant and is independent of the water current. Ball has also ascertained that both methods of conduction can operate at the same time. The latter method of conduction (*i.e.*, through the pith) is probably of the same nature as the "high-speed conduction" described for the petiole by Snow, due to the almost explosive contraction of highly turgid cells; the chief difference lies in the fact that high-speed conduction is through the phloem of the petiole.

Phototropic and geotropic phenomena have now been shown in all probability to be governed by hormone activity. The coleoptile of grasses has been much investigated with respect to its response to light, and a very large body of data has been accumulated on the subject. It will be convenient to consider this and cognate problems below.

### **Phototropism and the Growth Regulator of the Coleoptile**

As far back as 1882 de Candolle put forward the suggestion that the bending of an etiolated shoot towards incident light was caused by the fact that the illuminated side grew less than the side away from the light, and in this way a curvature towards the incident rays was developed. In other words, light had a direct effect on the plant and was directly responsible for the resulting curvature. On the other hand, Pfeffer considered that light acted as a releasing mechanism and that there was no quantitative relationship between the externally supplied energy and the resultant response of the plant. The stimulus in this instance, light, acted like the trigger of a gun. There is no quantitative connection between pulling the trigger of a gun and the resulting explosion. It does not matter whether the trigger be pulled quickly or slowly, vigorously or carefully, the force of the explosion

is always the same and depends on the amount of powder in the cartridge.

This conception of a stimulus affords the best explanation of the results obtained by Charles Darwin with the etiolated coleoptile of various grasses. Darwin discovered that the response of an etiolated coleoptile depends on the perception of the stimulus (in this case light) by a localised region of the organ *which is not the region of curvature*. This work has been confirmed again and again by numerous investigators. Curvature always takes place at the base of the coleoptile if the tip be illuminated. Hence, there must be a perceptive and a responsive region. As a matter of fact it has been discovered that only about 2 mm. of the apical region is sensitive. It is therefore evident that the excitation, whatever be its nature, is released at the tip and travels down to the motor region at the base and there brings about curvature.

Morphologically, the coleoptile is a cylindrical structure with two lateral veins. In 1896 Rothert cut the veins and discovered that the stimulus still passed when the tip was illuminated. This observation was confirmed by Fitting (1907). Boysen-Jensen (1910, 1913), however, obtained a different result. He ascertained that if in the air of the laboratory the vein away from the source of light were nicked there was no response, but if the vein towards the source of light were cut there was a positive response—*i.e.*, the coleoptile bent towards the light. He confirmed the observations of Rothert and Fitting that if the coleoptile were kept in a saturated atmosphere and the vein away from the light were severed there was still a positive response, and the same result was obtained if the vein were cut under water. Boysen-Jensen therefore concluded that the saturated atmosphere kept the gap full of sap, and the substance or substances responsible for the phototropic response of the coleoptile diffusing down from the apex could pass through the water-filled gap. If a mica plate were inserted on the side away from the light there was no response, while, if the mica plate were inserted on the side towards the light, curvature still took place. These observations have been confirmed by Purdie (1921) and Nielsen (1924).

Boysen-Jensen next cut a centimetre off the tip of the

coleoptile and then replaced it again with a gelatin base between it and the stump. He discovered that on illumination the coleoptile still responded. It is thus evident that the stimulus responsible for curvature can diffuse through gelatin.

Páal (1919) has made a number of interesting observations on coleoptile behaviour in unilateral light and considerably modified Boysen-Jensen's original technique. He has shown that none of the results obtained are due to stray light from the illuminated apex. A further experiment of Páal's in this connection is of importance. He removed the tips of coleoptiles of *Coix lacrima* and replaced them excentrically on the decapitated stumps in the way shown in Fig. 53. He found as a result that the coleoptiles curved away from the side covered by the tip, and he interpreted this as showing that a growth-accelerating substance was produced at the tip which passed across the moist discontinuity and then passed down the side of the coleoptile covered by it, with the result that this side grew faster than the other and caused the coleoptile to curve.

A series of experiments by Stark (1921) have shown that the tips of foreign genera placed on stumps also give a response.

Thus, for example, if the tip of an *Avena* coleoptile be placed on a stump of *Hordeum*, a response is still given, although he found that the reaction was much weaker in the majority of cases. In the particular case quoted here, *Avena* tip on *Hordeum* stump, the response was frequently much stronger than if the decapitated tip of a *Hordeum* coleoptile had been replaced on its own base. Stark also discovered that it did not matter whether the apex were accurately replaced, or even if it were replaced back to front; the response was always similar. Stark further tried the effect of placing extracts from wounded coleoptiles on the side of the stump, and found that a response occurred on the side on which the extract had been placed. Stark and Drechsel (1922) have also tried the effect of decapitating coleoptiles and illuminating

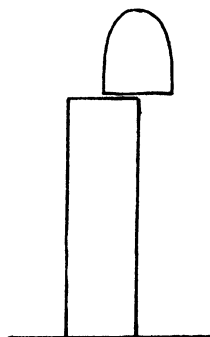


FIG. 53.

the tips with unilateral illumination. The tips were then carefully replaced on the stumps and it was discovered that a response was given in the direction of the original illumination of the tips. Brauner (1923, 1924) has attempted the reverse experiment. In this case the tips of the coleoptiles were removed and placed in the dark and the stumps exposed to unilateral light. The tips were then replaced on the stumps and the coleoptiles returned to the dark for four hours. It was found that twenty-one of the thirty-two coleoptiles experimented upon showed a curvature in the direction of the original illumination. The control plants, *i.e.*, those in which the tips were not replaced on the illuminated stumps, showed no response at all.

Went (1928) has carried out a particularly brilliant investigation on this growth hormone, using the coleoptile of the oat, and has definitely established the existence of some growth substance (Wuchstoff). Snow (1932a) has termed this substance or group of substances "growth-regulators," or simply G.R. ; it will be so referred to here. Went grew his coleoptiles in total darkness and all subsequent manipulations were carried out upon them in red light, which has no influence upon their reaction. In the first place the tips of the coleoptiles were removed and placed upon thin slices of jelly, either gelatin, agar, or silicic acid. He discovered that if a portion of this jelly were subsequently placed excentrically on the stump of another coleoptile from which the tip had just been removed, a negative curvature took place ; in other words, the coleoptile bent away from the side receiving the jelly. Went interpreted this result as showing diffusion of G.R. from the jelly down that side of the coleoptile. It also shows that G.R. diffuses from the coleoptile tip. At 25° C. curvature took place in 110 to 120 minutes, and the coleoptiles very regularly curved away from the side covered by the block of jelly containing the extract of G.R. This negative curvature is followed in about 170 minutes by a positive curvature, and is considered to be due to the regeneration of a new physiological tip, which, it would seem, is first formed on the side of the decapitated stump away from the block of jelly, and from there a fresh supply of G.R. is released into the base of the coleoptile. This "physiological regeneration

of the tip," as it has been called, was first described in 1896 by Rothert and confirmed by Söding (1923, 1925, 1929), who showed that after a time the uppermost region of the stump proceeds to make fresh G.R. Went has further shown that the amount of curvature is proportional to the number of tips that were originally placed upon the slice of jelly and to the period of time that they were allowed to remain upon it.

Went has suggested that the mode of action of G.R. is to modify the process of extension in cell size. It is clear that its method of action must be to increase cell extension and not cell division, for the cells in the elongating region of the coleoptile have ceased to divide. Went put forward the view that the walls of the cells become more plastic under the action of G.R. so that they suffer irreversible stretching beyond their limit of elasticity by the turgor pressure of the cell sap, and in this way the length of the cells is permanently increased. Heyn (1931) has strongly supported this view of Went. (See also Heyn and Overbeek, 1931.) Went also endeavoured to ascertain the mode of transport of G.R. down the coleoptile. He found protoplasmic streaming to be present in the cells of the coleoptile and considered this phenomenon to be responsible for the downward transport of G.R. He was even able to observe the transference of the substance from a piece of jelly at the upper end of a coleoptile stump down the coleoptile to another piece of jelly at the base. From rough diffusion experiments he was able to arrive at the approximate molecular weight of G.R. and found it to be about 376.

Van der Weij (1932) has made a number of observations on the problem of transport of G.R. in the coleoptile. Using G.R. prepared from urine (see below), he employed blocks of agar containing known concentrations of G.R. and placed them on the decapitated tops of coleoptiles, which rested on a block of pure agar, and tested the effect of (a) temperature, (b) initial concentration of G.R. (c) length of coleoptile, and (d) orientation of coleoptile on the transport of G.R. He measured the "velocity" of transport by the time taken for the first appreciable trace of G.R. to reach the lower block of agar and the "intensity" of transport by the amount of G.R. that subsequently reaches the

son's equation gives a more nearly correct representation of physiological processes than that of Mitscherlich.

G. E. Briggs, Kidd and West (1920), Vyvian (1924), and Briggs (1928) considered that the S-shaped curves of monomolecular reactions and the S-shaped curve obtained for the growth of a plant show but a superficial resemblance which is of no fundamental importance, and they held that the falling off in the growth rate per unit dry-weight was due to increasing differentiation into productive and non-productive tissue. Gregory (1928b) has replied in detail to these criticisms, and the originals should be consulted.

An important criticism has been brought forward by Snell (1929) against the autocatalytic theory of growth. He considered that the equations which these various workers have used cannot apply in the case of living organisms, because of the effect of increasing size on the concentration of the reagents involved in growth. The equations, based on the assumption that growth is of the nature of an autocatalytic reaction, hold true only on the condition that the volume occupied by the reacting substances remains constant, and since a growing organism is constantly increasing in volume this condition is not met. When a chemical reaction is carried out in the laboratory, the reagents are ordinarily dissolved in water or some solvent, and the volume of the solvent is kept constant throughout the reaction, and it is to such reactions carried out under such conditions that the usual formulæ based on the law of mass action are intended to be applied. Snell therefore considered that the equations for growth should include a volume term. Thus if  $A$  represents the concentration of the substrate,  $x$  the amount of the end-product at a time  $t$ ,  $V_0$  the volume of the organism at the beginning of the growth-cycle, then the corrected equation becomes :—

$$\frac{dx}{dt} = K_1Ax - K_2 \frac{x^2}{cx + V_0}$$

where  $c$  is a constant. Such an equation gives a sigmoid curve, but one of decidedly different form to that of Robertson, and, in fact, it is very doubtful if it can be applied to the majority of growth curves.

lower block per unit of time. He found that the "intensities" of transport through paths of different length are almost equal, and that the "velocity" of transport is nearly independent of temperature. He was able to show that the "intensity" of transport increases with increase in the initial concentration, whereas the "velocity" is apparently unaltered. Van der Weij is not in agreement with Went regarding protoplasmic streaming as a factor in transport of G.R. for, since the "velocity" of transport is nearly independent of temperature, it is difficult to see how protoplasmic streaming can play a part in the matter, for the latter has a high temperature coefficient.

### Geotropism and the Action of G.R. upon the Root

Snow (1928) ascertained that when the root tips of *Vicia Faba* were cut off at a distance of 2 mm. from the vegetative point and replaced with gelatin, geotropic curvature was still shown. When the tips were removed and the roots subsequently geotropically stimulated, practically no response was given. For example, in one experiment thirty-two roots were decapitated and then laid horizontally; out of this number, four gave a slight response and one a strong response, the remainder showed no curvature at all. It was also found that if the root tips were killed by boiling and replaced, no response was shown, so that the dead tip has no effect upon the gravitational response of the root. When, however, roots were decapitated and the tips replaced on the stumps with an intervening layer of gelatin, a number reacted to gravity. A decapitated root, then, becomes almost insensitive to geotropic stimulus, but its sensitivity can be in large measure restored by replacing the tip.

Cholodny (1924, 1926, 1928, 1929), working with *Zea Mays*, found that the sensitivity of decapitated roots to geotropic stimulus could be restored to a great extent by replacing their own tips, but, more remarkable still, this sensitivity to gravity was restored to an even greater degree when the tips of the coleoptiles were placed upon the decapitated stumps. The question naturally arises here: Why is it that the tip of the coleoptile, which restores

the sensitivity of the coleoptile itself to gravity (in this case positive geotropism is shown and the coleoptile curves upwards), brings about upward curvature of the shoot and downward curvature of the root ? According to Cholodny, this result is due to the different nature of the two stumps, and he considered that their growth-rates are oppositely affected by the same substance which diffuses into them from the coleoptile tip, the growth of the coleoptile being *accelerated* and that of the root *retarded*. Cholodny supported his view by finding that the rates of growth of decapitated roots and coleoptiles are affected in opposite directions whether root tips or coleoptile tips are placed upon them. He showed that the growth of roots is retarded, whereas that of coleoptiles is accelerated by both kinds of tip. It therefore appears that the root tip, like that of the coleoptile, excretes some substance which acts in the same way as that of the coleoptile. This explanation has been accepted by Keeble, Nelson and Snow (1929, 1931), who have been able to confirm Cholodny's original findings. They also found that if the tip from a root which had been previously geotropically stimulated were placed upon a stump which had not been stimulated in this manner, curvature followed. Response was also found when the tip of an unstimulated root was placed on a root which had been first decapitated and its stump geotropically stimulated. Keeble, Nelson and Snow consider that the coleoptile tip can bring about geotropic response in the root in two ways. In the first place, a geotropic stimulus can be transmitted from the tip to an unstimulated stump, and secondly, from an unstimulated tip some influence is transmitted to the stump which makes it also sensitive to a geotropic stimulus. According to these investigators, the root tip acts in the first of these two ways and possibly in the second way as well. Went (1926) considered that the geotropic curvature of the coleoptile, when it is placed in a horizontal position, is due to the G.R. from the tip in some way becoming redistributed, so that its concentration on the lower side of the elongating region is higher than on the upper side, with the result that upward curvature takes place. According to Keeble, Nelson and Snow, if it be admitted that the redistribution of G.R. can take place

both in the tip as well as in the elongating region, the two ways in which the replaced tips affect the stumps can be understood. "Firstly, from a geotropically stimulated tip the growth substance diffuses out in unequal concentrations on the two sides, and so causes an unstimulated stump to curve. Secondly, from an unstimulated tip it diffuses out on all sides equally; but on passing into a stump which has been geotropically stimulated, it is there somehow redistributed so as to reach the upper and lower sides in different concentrations. The fact that the root stump, with coleoptile tip upon it, curves downwards and not upwards should probably be interpreted in the way suggested by Cholodny, who found that it is *retarded* in rate of growth by the coleoptile tip, although the coleoptile stump is accelerated."

Cholodny has taken up a similar standpoint and considered that

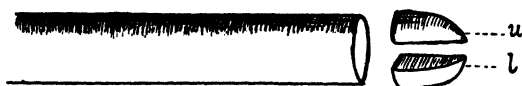


FIG. 54.—Method of preparing half tips for extraction of G.R.  
 $u$  = half which was uppermost during stimulation,  $l$  = half which was lowest during stimulation. (After Hawker, *New Phyt.*)

the G.R. formed in the tips of either coleoptile or root is normally transmitted to the elongating regions in straight lines and reaches there in equal concentrations on all sides. But under geotropic stimulus the usual method of transport is in some manner diverted so that they travel obliquely and reach a higher concentration on the lower sides of the elongating portions of these organs.

The question of redistribution of G.R. has been exactly determined by Hawker (1932). Seeds of *Vicia Faba* were germinated, and when the roots were 6 to 12 cm. long two-thirds the number were stimulated in a horizontal position. The remainder were placed with the roots vertical. At the end of this period the tips of the stimulated roots were removed and divided into two halves, as shown in Fig. 54. The divided root tips were then placed on small blocks prepared from gelatin, four to each block. The tips were allowed to stand on the blocks for one hour in order to allow the G.R. to diffuse out of them into the gelatin. At the end of

this time the roots, which had been in a vertical position, were decapitated and the small blocks of gelatin were placed upon the stumps excentrically. In this way half the roots received blocks of gelatin into which G.R. from the lower halves of the stimulated tips had diffused (A), and the other half received blocks into which G.R. from the upper halves of the stimulated tips had diffused (B). The results of this experiment are given below :—

	Number of Seedlings used.	Percentage Response.	Average Curvature.
(A) Gelatin containing G.R. from lower halves of tips . . . .	24	91.6	30.1
(B) Gelatin containing G.R. from upper halves of tips . . . .	24	66.6	10.9

It is clear from these figures that G.R. had accumulated to a greater extent on the lower side of the stimulated tip. The response shown in all cases was towards the block of gelatin, a fact which gives added evidence for the view that G.R. retards the growth of roots.

As Hawker has pointed out, these results at first sight are directly opposed to the statolith theory of geotropism. Under the conditions of a growth-regulating substance diffusing out equally from all sides of an unstimulated root tip and becoming redistributed in the stimulated stump, it is obvious that perception and response can take place without any displacement of statolith starch in the root cap. In *Zea Mays*, for example, no starch is to be found in the growing region of the root, and here the geotropic sensitivity of the growing zone demonstrated by Kēeble, Nelson and Snow must be due to some other perception mechanism than movable starch grains.

Hawker has made some interesting observations on geotropic presentation time. Experiments were carried out in which roots of *Vicia Faba* were stimulated prior to decapitation, and their tips were then removed and replaced so that the side of the tip which had been lowest during stimulation was now opposite the

stump which had been lowest. The roots were then placed in a vertical position. By actual experiment the geotropic presentation time for roots of the particular strain of *V. Faba* used in this work (Early Long Pod) at a temperature of between 10° to 15° C. was found to be about one hour. It was discovered that if the roots were stimulated for between one and one and a quarter hours and the tips were then reversed in the manner described above, curvature usually took place towards the side of the tip that was lowest during stimulation. On the other hand, if the stimulation was continued for a greater period than one and a quarter hours, curvature was usually towards the side of the stump that had been lowest during presentation time, so that conduction of G.R. must have taken place before decapitation. These results obtained by Hawker suggest that presentation time is the period necessary for the perception of the stimulus by the tip, and that the conduction of G.R. backwards from tip to stump does not take place until later, so presentation time is the time necessary for the redistribution of sufficient G.R. in the tip to cause curvature of the growing zone.

### The Chemical Nature of G.R.

It was discovered by Nielsen (1930) that the fungus *Rhizopus suinus* excretes into its culture medium a substance which markedly accelerates the growth of coleoptiles and retards the growth of roots. Later (1931a), Nielsen showed that yeast also contains such a substance, while Boysen-Jensen (1931a, 1931b) has ascertained the presence of such a substance in various bacteria as well as the ascomycete, *Aspergillus niger*. It is possible that all these substances are one and the same, or, at any rate, very closely akin chemically. They are soluble in ether, so they can be extracted with this reagent. On the other hand, Nielsen (1931b, 1932) has been able to show that *Rhizopus suinus* elaborates another substance which possesses the power of increasing the weight of *Aspergillus niger*. This substance is insoluble in ether.

Kögl and Haagen-Smit (1931) have obtained G.R. from various sources : yeast, *Rhizopus suinus*, coleoptile of *Zea Mays* and urine.

From urine they obtained a crystalline compound, molecular weight between 330 to 353, and approximate formula of  $C_{20}H_{38}O_4$ , which produced curvature in *Avena* coleoptiles. Kögl and Haagen-Smit have introduced the term "Avena-Einheit" (*Avena* unit = A.E.), in order to compare the strengths of different sources of G.R. A unit of A.E. is defined as being the amount of G.R. contained in a block of 3 per cent. agar of dimensions  $2 \times 2 \times 0.5$  mm., which brings about a curvature of  $10^\circ$  in two hours at a temperature of  $22^\circ$  to  $23^\circ$  C. when placed excentrically on one decapitated coleoptile of *Avena*. They showed that the crystalline product they had isolated from urine had an efficiency of 30,000,000 A.E., i.e., 1 mg. of this body could impart a curvature of  $10^\circ$  to 30,000,000 *Avena* coleoptiles. The figures they obtained for the molecular weight (330 to 353) agreed fairly closely with the approximate value of 376 obtained by Went from diffusion experiments. It was found that the substance contained no sulphur, phosphorus or nitrogen, and appeared to be an aliphatic organic derivative.

Seubert (1925) has shown that malt extract contains large amounts of G.R., but according to Dolk and Thimann (1932) different commercial samples of malt vary considerably with respect to the amount present. Dolk and Thimann have carried out an elaborate chemical investigation on the nature of G.R. the source of their product being *Rhizopus suinus*. The G.R. was extracted with specially purified ether. It was found that unless the ether was carefully purified by redistillation over ferrous sulphate and lime there was considerable loss of activity in the G.R. For comparative purposes, Dolk and Thimann employed the following unit system: A unit was chosen as that quantity of substance which has to be present in 1 c.c. of solution, to give, after mixing with 1 c.c. of agar (8 per cent.), an angle of curvature of  $1^\circ$  to an *Avena* coleoptile. The total number of units per c.c. of solution is then found by multiplying the angle measured by the dilution in which the test was carried out.

It was found that the active solutions of the substance were somewhat acid ( $pH = 4.0$ ). On the addition of alkali and extraction of the alkaline solution with ether, the ether extract was found

to be inactive. The G.R. was not destroyed by alkali, for the remaining aqueous layer was highly active. It is clear that the formation of a salt has taken place with an accompanying loss of solubility in ether. G.R. must therefore be considered as an acid. The dissociation constant was found to be  $1.8 \times 10^{-5}$ . The substance showed marked susceptibility to oxidation, and its activity was rapidly destroyed by alkaline permanganate ( $\text{KMnO}_4 = 0.0024\text{M}$ ). It reduced silver nitrate, but Fehling's solution had no action upon it. An attempt was made to prepare the oxime with hydroxylamine, but the results proved to be inconclusive, so that it is doubtful if an aldehydic group is present in the molecule. On the other hand, the extreme sensitivity of the substance to oxidation indicates that double bonds of some kind must be present. The presence of acid, in this case  $2\text{N.HCl}$ , after three hours' standing reduced the activity of G.R. by half. With  $\text{N.HCl}$ , destruction of activity proceeds slowly and requires more than thirty minutes at  $100^\circ \text{C.}$ , and more than three hours at  $25^\circ \text{C.}$

### Growth and Inhibition

It is a well-known fact that if the apices of many seedlings of monopodial growth are removed the axillary buds grow out and form new shoots. The apex apparently exerts some influence which prevents the development of the axillary shoots. The suggestion has been made that an inhibitory growth substance is formed at the stem apex and is passed down to the shoots which prevents the development of the axillary buds. Another suggestion is that the growing apex in some way draws to itself the supply of nutrient fluid or perhaps some particular substance necessary for growth, and the axillary buds are in this way kept in a dormant state through starvation. Loeb (1924), after a number of investigations on regeneration in *Bryophyllum calycinum*, considered that inhibition in this plant might be due to a lack of necessary nutrient material, although he did not exclude other causes.

Snow (1925b, 1929a, 1929h, 1931, 1932b) has critically examined

Loeb's work, and is of the opinion that the transport of an inhibitory substance of hormone from the apex is the true explanation. Using *Phaseolus multiflorus* and *Vicia Faba*, he was able to show that ringing of the epicotyl, so that all tissue, including the cambium, was removed external to the wood, still allowed of the passage of the inhibition. The control plants which were decapitated and unringed showed signs of regeneration after four days, whereas the treated plants showed no axillary growth at all. It is thus evident that the inhibition can pass through a stem that has been ringed. He also discovered that the inhibition could pass through the pith, although it is considerably weakened in its effects. When the main apex and axillary bud were only connected by the xylem with a few cells of pith parenchyma adhering to it, the inhibition again passed down. In a second series of experiments a zone of the stem of *Vicia Faba* was killed by a jet of steam playing on it for twenty to thirty seconds. Although only the outer cortical cells were killed by this treatment, the inhibition failed to pass owing to "physiological shock," and the axillary bud grew out below the treated zone, the main stem continuing its growth. When the cut surfaces of tissues of seedlings were bound together in pairs and each pair was decapitated, the inhibition was found to be unable to pass across from one to the other. The question as to whether the inhibition could or could not pass across a watery gap proved difficult to show experimentally. The difficulty, however, was surmounted by splitting seedlings of *Phaseolus multiflorus* longitudinally through the lower region of the epicotyl, cotyledons, hypocotyl and main root, and binding the parts together. The inhibition was found to pass through the watery gap.

To test whether this inhibition passed upwards in the transpiration stream, young seedlings of *Vicia Faba* were decapitated in the epicotyl, so that the two axillaries of the cotyledons grew out. Those seedlings were then selected in which the axillaries were of nearly equal strength, and the shorter of the two was then decapitated above its second leaf and a bud was allowed to remain in the axil of one of the two leaves of this shoot, while the bud in the axil of the other was removed. The remaining bud on

the shoot showed scarcely any growth even after three or four weeks. Presumably, therefore, it was inhibited by the action of the apex of the longer shoot, for if this were also decapitated, then the bud grew out strongly. A zone of tissue (about 4 mm. long) was now killed with a hot glass rod in such a way as not to char the tissues or allow the cell contents to boil, and the dead zone was painted over with vaseline. The controls, in which the apex of the longer shoot was also removed as well as the axillary buds, save the one to be measured, showed a mean growth of 3.37 mm.  $\pm 0.25$  compared with 1.54 mm.  $\pm 0.13$  for the treated plants. The difference of the mean is 1.83, which is 4.36 times its own standard deviation, and therefore fully significant.

The interpretation placed by Snow on these experimental results is that an inhibitory substance formed at the growing apex of the longer shoot is transported through the living tissues and leaks into the xylem just below the dead zone. It is then drawn up with the transpiration stream through the dead zone and leaks out into the living tissue of the shorter shoot, where it inhibits the growth of the axillary bud.

It is desirable to know exactly what part of the apex of the shoot brings about inhibition of the axillaries. Snow (1929b) has endeavoured to determine this point. It is known that in certain plants the larger leaves can also bring about inhibition. Snow employed *Pisum sativum*, *Vicia Faba* and *Phaseolus multiflorus* in his investigation. In *P. sativum*, which was the principal species used, the leaves are arranged in two ranks (distichous), and it was discovered that the axillaries of the first leaves could be made to grow out by removing the leaves from the stem from below upwards to those of about 2.5 mm. in length, inclusive. In seedlings of *V. Faba*, the first leaf axillary of the main shoot, which is a very large bud and grows out readily, grew out slowly when leaves were removed only so far as to those of length from 8 to 10 mm., while in two seedlings of *P. multiflorus* the axillaries of the cotyledons were made to grow out rapidly by removing the leaves of those up to 1 to 2 mm. long, inclusive, and also removing the higher buds. Yet in all these plants the apices of the stems and very small remaining leaves were not injured but went on

growing. In order to keep the axillaries growing indefinitely it was necessary to keep on removing the largest leaves of the terminal bud after their growth intervals. When this was not done, the growth of the axillaries came to a standstill before long. In the circumstances it must be concluded that a considerable part at least of the inhibiting effect of the intact shoot comes from some of the leaves that have reached more than a certain small size. When these are removed the axillaries grow out until the remaining very small leaves near the apex grow to the necessary size and once more inhibit their growth completely. The strength of this inhibiting power is not constant in amount for any given size of small leaf, but increases with the age of the seedlings, and consequently with the height of the leaf on the plant. In very young seedlings of *Pisum sativum*, only 20 mm. high, inhibition is very much weaker. Snow ascertained that a single leaf never, or scarcely ever, inhibits completely, so that in a normal shoot complete inhibition must be due to the action of several leaves acting in concert together, but the partial inhibiting effect of a single leaf increases slowly to a maximum with increase in its length and then falls away, until in its final size its inhibitory power is practically nil. Although the stem apex and youngest leaf primordia of length 1 mm. or less do not inhibit appreciably, it is nevertheless not wholly inaccurate to speak of the stem apex as the inhibiting region, for almost the entire inhibitory effect of the shoot, for example, in *Pisum sativum*, comes from three of its leaves—i.e., those between 2 to 6 mm., 6 to 18 mm., and 18 to 30 mm. long respectively in each growth interval—and, of these three leaves, two still form part of the terminal bud, and the third is only a small way beneath it.

It has also been shown by Snow (1931) that in seedlings of *Pisum sativum*, the strength of the inhibition increases with the length of the intervening stem. If young pea seedlings be decapitated in the epicotyl, plants can be obtained with two shoots springing from the axils of the cotyledons. Snow has examined the question of how it is that when these shoots are of nearly equal length they do not inhibit one another, for if they be not of equal length, the weaker shoot soon stops growing and after

O. W. Richards (1928) found that the growth curve of yeast was an asymmetrical S-shaped curve. Increase in growth in this particular case was measured as the increase in the number of cells in a unit volume. Robertson's expression only applies to symmetrical S-shaped curves, and, since the curve here is asymmetrical, it must be concluded that yeast growth is not limited by a monomolecular autocatalytic reaction. From a consideration of the equations of multimolecular reactions, and making the assumption of a slowest limiting master reaction, Richards concluded that this master reaction for yeast was of five molecular complexity.

The average plant in its natural surroundings is exposed to a large and ever-changing variety of external factors such as variations in temperature, rainfall, light intensity, etc., yet in spite of these continually changing conditions it manifests a steady rate of growth till it reaches maturity. The plant is a complex physico-chemical system composed of co-ordinated chemical reactions, and these co-ordinated reactions no doubt conform to Le Chatelier's theorem which states: "When one or more of the factors determining an equilibrium is altered, the equilibrium becomes displaced in such a way as to neutralise as far as possible the effect of the change." Action and reaction are continually taking place in the plant, and every change in the surrounding environment must be met by a corresponding response in the plant.

### Temperature

Since both the physiological processes of carbon assimilation and respiration are accelerated by a rise in temperature, and again, since assimilation is always in excess of respiration under normal conditions of metabolism, it follows that an increase of temperature will lead to an increase of growth. The range of temperature suitable for growth is restricted. Too high a temperature leads to a collapse of the metabolic machine and brings about the death of the plant, and, similarly, if the temperature be too low, the metabolic rate is very considerably lowered and growth is brought to a standstill.

some weeks dies. This latter result is due to the fact that the stronger shoot inhibits the growth of the weaker, because, if the shoot of stronger growth is removed, the weaker proceeds to grow indefinitely. It was found that in seedlings in which the lateral shoots were of nearly equal length, if one were defoliated from below upwards until only the very small leaves at the apex were left, the defoliated shoots grew very much less than the intact shoots, and finally ceased growing altogether and died, whereas, in the controls in which one shoot was defoliated and the other completely removed, growth continued. It is clear from these results that defoliation of the kind described does not arrest the growth of a pea shoot if it be the only shoot on the plant. If, however, there be another intact growing shoot present on the same plant as the defoliated one, the latter is eventually killed. It would therefore seem that when two intact and equal cotyledonary axillaries are present on the same plant they are in a sense in equilibrium. If one of them be defoliated the balance is upset and it is rapidly inhibited and killed by the other. It was discovered that it mattered not whether the plants were grown in a good light or in weak light, and that the inhibitory effect came from the removal of the rapidly developing young leaves, which are also the inhibitory members, so that this inhibitory action is not due to lack of carbohydrates owing to the removal of the leaves on the defoliated shoot. Snow at first considered that the inhibitory influence that comes from the leaves is of a "polar" nature, so that two such influences coming from two cotyledonary shoots from opposite directions in some way counteract one another. If one of the shoots be weakened in any way by such a process as defoliation, the inhibitory influence from the intact shoot travels up it, inhibits its growth and finally kills it. He later (1982b) found this conclusion to be erroneous.

It was further found that as far as the killing of the fully grown parts is concerned, the difference in fate between the two shoots is somehow due to their being differently orientated with regard to the influence coming from the developing leaves; for the lower parts of the intact shoots which remain quite healthy are in a line between the developing leaves and the roots, and the

influence coming from these leaves travels downwards basipetally, whereas, the defoliated shoot, which is ultimately killed, is not in this line, and further, in it the same influence travels in an upward direction. That this is a possible explanation is shown by the following experiment: Seedlings of *Pisum sativum* with several elongated internodes were decapitated in an upper internode and then defoliated. Two axillary buds were allowed to remain on the main shoot, and it was found that as a general rule one of these took the lead and grew out more strongly than the other to form a new shoot. The internodes left above the freshly developing shoot after about a month turned yellow and died from the top downwards to a level of from 5 to 10 mm. above the origin of the axillary shoot, and this remaining zone remained alive for a considerable time, but in time it too also died. The region of the main stem below the axillary shoot remained green and healthy indefinitely. There is therefore no occasion to postulate that the main stem possesses some special kind of susceptibility to inhibition differing in kind from the axillary shoot, for the lower part of the main stem, which is in the line between the developing leaves of the axillary shoot and the roots, and in which the influence is travelling basipetally, remained healthy, as did the axillary shoot itself. It was only the part of the main stem which was not in this line, and in which the influence travelled acropetally that was killed.

The question arises here: Why is it that the leaves near the apex of the shoot of a Leguminous seedling inhibit the growth of axillary buds and also tend to inhibit the growth of other shoots and subsequently kill them, yet at the same time these leaves promote the elongation of their own shoots beneath them and stimulate their growth in thickness?

It was shown as far back as 1898 by Jost that as a general rule (although there are exceptions) growth in stems only takes place under the influence of leaves that are still actively growing and that the influence from these leaves only travels in the *downward* direction. Karstens (1924) has put forward the suggestion that this "cambial stimulus," as it has been called, is a hormone. The problem has been critically re-examined by Snow (1932b).

Working with *Vicia Faba*, Snow found that the question of the orientation of the defoliated shoots with regard to the growing leaves of intact shoots, and the further fact that if the defoliated shoot be out of line with the intact shoot and roots is only a matter of subordinate importance for the parts that receive the cambial stimulus, for they survive and grow about equally fast in either position. Snow has been able to confirm the fact that this cambial stimulus only travels in the downward direction from the leaves. According to him the answer to the question as to why young leaves near the apex stimulate the growth of their own shoot beneath them, but inhibit lateral buds or shoots, is that the young leaves transmit in a downward direction a variety of growth-promoting influences which override their inhibiting influence in the parts which they reach, but do not enter lateral shoots or buds, whereas the inhibiting influence is able to penetrate these organs and stop their growth. The cambial stimulus cannot penetrate into lateral shoots or buds, because it is apparently unable to travel in an upward direction, but it is able to protect the mature parts of the shoot from being killed. The growing leaves also protect the young internodes of their own shoot from being inhibited in their elongation, and it is probable that they are able to do so partly or mainly by means of a stimulus which only appears to be able to travel for a short distance.

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According to Leitch (1916), the effect of temperature on the growth rate of *Pisum sativum* is shown by a uniform curve for the range of temperature—2° C. to 29° C. (Fig. 47)—which closely resembles the curves obtained for respiration by Kuijper. Above 29° C., marked fluctuations make their appearance, and no single curve can express the relationship between growth-rate and temperature. Hence, for each rise in temperature a separate curve must be employed to express the rate of growth in successive

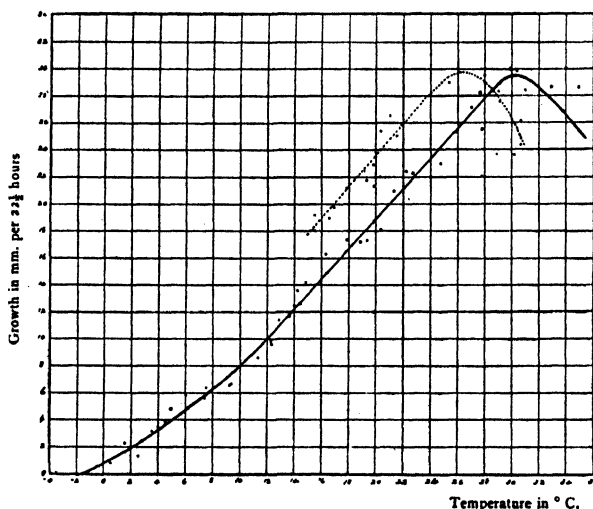


FIG. 47.—Curves illustrating the effect of temperature on the growth of roots of pea seedlings between 2° C. and 36° C. (After Leitch, *Ann. Bot.*)

periods of time. F. F. Blackman's "time-factor" is of importance here. At 30° C. and 35° C. the rate of growth in the first ten minutes is the highest attained, while in the first half-hour there is a rapid fall, which in turn is succeeded by a second maximum showing recovery, and thereafter there is a gradual fall (Fig. 48). At 40° C. decrease in growth is rapid and uniform, and no recovery is registered. The coefficient for a rise in temperature of 10° shows a distinct falling off as the temperature increases, and from Leitch's results it is only between 10° C. and 20° C. that the

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